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Public Health Department

Notification

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Government of India, Ministry of Health, Family Planning, Works, Housing and Urban Development (Dept. of Health), Notification No. F.1-6/62-D dated 2nd July, 1969 published in the Gazette of India, Part II Section 3 sub-section (ii) is hereby republished for general public information.

V. R. Vaze, Under Secretary (Health).

Panaji, 30th October, 1969.

Notification

In exercise of the powers conferred by sections 12 and 33 of the Drugs and Cosmetics Act, 1940 (23 of 1940), the Central Government, after consultation with the Drugs Technical Advisory Board, hereby makes the following rules further to amend the Drugs and Cosmetics Rules, 1945, the same having been previously published as required by the said sections, namely: —

1. These rules may be called the Drugs and Cosmetics (Third Amendment) Rules, 1969.

2. In the Drugs and Cosmetics Rules, 1945 (hereinafter referred to as the said Rules), the existing rule 3A shall be renumbered as sub-rule (1) thereof and after sub-rule (1) as so renumbered, the following sub-rule shall be inserted, namely: —

"(2) The functions of the Laboratory in respect of the following drugs or classes of drugs shall be carried out at the Indian Veterinary Research Institute, Izatnagar or Mukteshwar and the functions of the Director in respect of the said drugs or classes of drugs shall be exercised by the Director of either the said institutes.

(1) Anti-sera for veterinary use.

(2) Vaccines for veterninary use.

(3) Toxoids for veterinary use.

(4) Diagnostic Antigens for veterinary use".

3. In rule 31 of the said Rules, the following proviso shall be inserted, namely: —

"Provided that in the case of biological and other special products intended for veterinary use the standards of strength, quality and purity, if any, shall be those that are specified in Schedule F(I) and the tests prescribed in that Schedule shall be applicable for determining whether any such imported drug complies with the said standards and where no standards are specified in Schedule F(I) for any veterinary drug, the standards for such drug shall be those specified in the current edition, for the time being in force, of the British Veterinary Codex".

4. For rule 32 of the said Rules, the following rule shall be substituted, namely: —

"32-Packing and labelling of imported drugs: — No drug shall be imported unless it is packed and labelled in conformity with the rules in Parts IX and X and Schedule F and further conform to the standards laid down in Part XII provided that in the case of drugs intended for veterinary use, the packing and labelling shall conform to the rules in Part IX and X and Schedule F(I)".

5. In rule 44 of the said Rules, after the First proviso, the following proviso shall be inserted, namely: —

"Provided further that for the purpose of examination of Antisera, Toxoid and Vaccines and Diagnostic Antigens for Veterinary use, the person appointed shall be a person who is a graduate, in Veterinary science, or general science, or medicine or pharmacy and has had not less than three years' experience in the standardisation of biological products".

6. In rule 49 of the said Rules, after the first proviso, the following proviso shall be inserted, namely: —

"Provided further that only Inspectors who are graduates in veterinary science or medicines or general science or pharmacy and have had not less than three years' experience in the manufacture or testing of biological products shall be authorised to inspect the manufacture of veterinary biological products".

7. In rule 76 of the said Rules after the proviso to condition (1) (relating to supervision by competent Technical staff), the following proviso shall be inserted, namely: —

"Provided further that for the drugs specified in Schedule C and schedule C(1) meant for veterinary use, the wholetime employee under whose supervision the manufacture is conducted may be a graduate in Veterinary science or general science or medicine or pharmacy of a University recognised by the Central Government and who has had at least three years' experience in the manufacture of biological products".

8. In rule 78 of the said Rules, after the word and letter "Schedule F", the following shall be inserted, namely: —

"or Schedule F(I) as the case may be,".

9. In rule 97 of the said Rules, for sub-rule (3), the following sub-rule shall be substituted, namely:—

"(3) The container of a medicine made up ready only for treatment of an animal shall be labelled conspicuously with the words 'Not for human use; for animal treatment only' and shall bear a symbol depicting the head of a domestic animal.";

10. In rule 107 of the said Rules, for the Explanation the following "Explanation" shall be substituted, namely: —

"Explanation: — For the purpose of this rule the expression "proper name" means the proper name stated in Schedule F or if no such name is stated, the name descriptive of the true nature and origin of the substance; Provided that in the case of veterinary biological product the expression "proper name" means the proper name stated in Schedule F(1) or if no such name is stated, the name or synonym given in the current edition for the time being of the British Veterinary Codex, or, if no such name is stated either in Schedule F(1) or the British Veterinary Codex, the name descriptive of the true nature and origin of the substance approved by the Licensing Authority".

11. In rule 108 of the said Rules, in sub-rules (3) and (4), after the word and letter 'Schedule F', the following words, letter, brackets and figure shall be inserted, namely: —

"or in Schedule F(I), as the case may be,".

12. In rule 109 of the said rules —

(*a*) in sub-rule (1), after the words and letter 'Schedule F', at both the places where they occur, the following words, letter, brackets and figure shall be inserted, namely: —

"or Schedule F(I), as the case may be".

(*b*) in sub-rule (3) —

(i) after the words and letter 'Schedule F' where they occur first, the following words, letter, brackets and figure shall be inserted, namely: —

"or Schedule F(I), as the case may be";

(ii) for clause (*b*), the following clause shall be substituted, namely: —

"(*b*) the date on which the manufacture of the particular batch from which the substance in the container is taken was completed as defined in Schedule F or Schedule F(1) or if there is no definition in Schedule F or Schedule F(1) as hereafter defined in this rule, and in the case of vaccines prepared from concentrates, the date of completion of the final products and the bottling for issue".

13. In rule 111 of the said Rules, after the words and letter 'Schedule F', the following words, letter, brackets and figure shall be inserted, namely: —

"or Schedule F(1), as the case may be".

14. In rule 112 of the said Rules, after the word and letter 'Schedule F', the following words, letter, brackets and figure shall be inserted, namely: —

'or Schedule F(1), as the case may be'.

15. In rule 122 of the said Rules: —

(*a*) in clause (a) after the word and letter 'Schedule F', the following words, letters, brackets and figure shall be inserted, namely: —

'or Schedule F(1), as the case may be'

(*b*) in clause (*b*) after the word and letter 'Schedule F' the following words, letters and brackets and figure shall be inserted, namely: —

'or Schedule F(1), as the case may be'

(*c*) for clause (*c*), the following clause shall be substituted, namely: —

"(*c*) The substance shall conform to the standards of strength, quality and purity specified in Schedule F or Schedule F(1), as the case may be, and the tests for determining the strength, quality and purity of the substance shall be those specified in Schedule F or Schedule F(1) as the case may be";

(*d*) in clause (*d*) after the word and letter, "Schedule F" the following words, letter, brackets and figure shall be inserted, namely: —

'or Schedule F(1), as the case may be'

16. After rule 124 of the said Rules, the following rule shall be inserted, namely: —

"124-A. **Standards for veterinary drugs:** — For drugs intended for veterinary use, the standards shall be those given in the current edition for the time being in force of the British Veterinary Codex".

17. In Schedule D of the said Rules, items 2 and 3 along with their entries shall be omitted.

18. In the said Rules, after Schedule F, the following Schedule shall be inserted, namely: —

"SCHEDULE, F (1)

PART I — VACCINES

(A) *Provisions applicable to the production of bacterial vaccines.*

1. **Definition:** — (1) This part of the Schedule applies to bacterial vaccines made from any micro-organism pathogenic to man or other animal and to vaccines made from other micro-organisms which have any antigenic value.

(2) For the purposes of this part of the Schedule, a bacterial vaccine means a sterile suspension of a killed culture of the micro-organism from which the vaccine derives its name or a sterile extract or derivative of a micro-organisms, or a pure suspension of living micro-organisms which have been previously made avirulent.

2. **Staff of Establishment:** — A competent expert in bacteriology with sufficient experience in the manufacture and standardisation of biological products shall be in charge of the establishment responsible for the production of bacterial vaccine and he shall be assisted by a staff adequate for carrying out the tests required during the preparation and standardisation of the vaccines.

3. **Proper Name:** — The proper name of any vaccine shall be the name of the micro-organism from which it is made followed by the word «Vaccine» unless this Schedule otherwise provides or if there is no other special provision in this Schedule, some other name as approved by the licensing authority; provided that in the case of the under-mentioned preparations the proper name of the vaccine may be as follows: —

1. Anthrax Spore Vaccine (Living).
2. Blackquarter Vaccine.
3. Enterotoxaemia Vaccine.
4. Fowl Cholera Vaccine.
5. Haemorrhagic Septicaemia Adjuvant Vaccine.
6. Haemorrhagic Septicaemia Vaccine (Broth).

4. **Records:** — Cultures used in the preparation of vaccine before being manipulated into a vaccine, should be thorougly tested for identity by the generally accepted tests applicable to the particular micro-organism.

The permanent records which the licensee is required to keep shall include amongst others, a record of the origin, properties and characteristics of the cultures.

5. **Combined Vaccines:** — Vaccines may be issued either singly or combined in any proportion in the same container. In the case of combination of vaccines, a name for the combined vaccine may be submitted by the licensee to the licensing authority, and if approved, may be used as the proper name of the vaccine.

6. **Preparation:** — Bacterial vaccines, simple or polyvalent, are prepared from selected cultures after careful examination for their identity, specificity, purity and antigenicity. They may be prepared in the following manner: —.

(a) *Formal Cultures or Bacterins:* — The selected pure culture strain or strain are grown in a suitable fluid medium, at an optimum temperature, for an appropriate period. The pure growth is then exposed to the action of solution of Formaldehyde I. P. in suitable concentration and temperature. The product is finally filled in suitable strilised containers which are subsequently sealed.

(b) *Vaccine of Bacterial Products or Bacterial Derivatives:* — These vaccines are prepared by goowing the organisms on suitable media and then deriving specific antigenic constituents of the bacteria by various special methods.

(c) *Living Bacterial Vaccines:* — They are prepared from non-pathogenic but fully immunogenic strains of microorganisms. Strict aseptic precautions are taken throughout the preparation against the introduction of microbial contaminants.

7. **General Standard:** —

(a) *Description:* — Bacterial vaccines are colourless to yellowish brown liquids containing dead or viable bacteria in homogeneous suspension.

(b) *Identification:* — All types of vaccines confer active immunity in the susceptible animals which can be demonstrated by injecting suitable experimental animals with the calculated doses of the product and subsequently determining the presence of the protective antibodies in their serum and/ /or by challenging the vaccinated animals by injecting virulent strain of the homologous organisms. The protected animals should survive the challenge.

(c) *Tests for Sterility:* — All bacterial vaccines shall be tested for sterility in accordance with the provision of Rules 115 to 119 (both inclusive). If the vaccine contains added bactericide or bacteriostatic, a quantity of medium sufficient to render the growth inhibitor ineffective is added to the sample, or a suitable substance is added to the sample, or a suitable substance is added in a concentration sufficient to render the growth inhibitor ineffective but not itself to inhibit the growth of micro-organism.

(d) *Purity Tests for Living Bacterial Vaccines:* — Petri--dishes containing suitable meditable media are streaked with the final product and incubated at 37.C for 72 hours. The vaccine passes the test if no growth of micro-organisms other than those from which the vaccine was prepared, is observed. Other tests include examination for motility of the organisms, fermentation, reactions and thermoagglutination test and dye-inhibitior tests in case of bruceliza vaccine.

(e) *Safety Test:* — The safety of the vaccine shall be assessed by injecting it in appropriate does in suitable susceptible animals. No animal should show any untoward, general or local reaction, within seven days after inoculation.

(f) *Potency Test:* — Wherever applicable, susceptible experimental animals are inoculated with the calculated doses of the final product. The animals are challenged, after the period of immunisation, with virulent infective dose of the homologous culture along with the controls. The potency of the Vaccine is assessed by the survival of the vaccinated animals and the death of the controls.

8. **Labelling:**

(a) The label on the ampoule or the bottle shall indicate.

(i) Proper name.
(ii) Contents in millilitres or doses.
(iii) Potency, if any.
(iv) Batch number.
(v) Expiry date.

(b) The label on the outside container shall indicate.

(i) Proper name.
(ii) Contents in millilitres or doses.
(iii) Batch number.
(iv) Date of manufacture.
(v) Manufacturing licence No.
(vi) Manufacturer's name and address.
(vii) «For animal treatment only».
(viii) Storage conditions.

9. **Storage:** — Bacterial vaccines shall be stored, protected from light at temperature between 2.C to 4.C and shall not be frozen.

10. **Date of Manufacture:** — The date of manufacture shall be, unless otherwise specified in the individual monograph in this Part, as defined in clause *(b)* of sub-rule (3) of rule 109.

ANTHRAX SPORE VACCINE (LIVING)

1. **Synonyms:** — Airulent Anthrax Spire Vaccine or Bacillus Anthracis Vaccine (Living).

2. **Definition:** — The vaccine is a suspension of living spores of an uncapsulated avirulent strain of B. anthracis in 50 per cent glycerine saline.

3. **Preparation:** — Avirulent B. anthracis of known antigenicity is grown on suitable medium at PH. 7.4 in Roux flasks. After 72 hours incubation at 37.C, the pure sporulated culture growth which shows 70 to go per cent sporulation is washed with normal saline and glycerinated to the extent of 50 per cent by weight of the culture washing and the whole suspension is kept at room temperature for twentyone days to allow for the stabilization of the spores.

4. **Standard:** —

(a) *Description:* — It is slightly opalescent or pale brown semi-viscous liquid.

(b) *Identification:* — Uncapsulated B. *anthracis* which is avirulent can be isolated from the vaccine.

(c) *Sterility Test:* — Should comply with the test for sterility described in the general monograph on «Bacterial Vaccine».

(d) *Purity Test:* — Complies with the «purity tests for living bacterial vaccine» described under the general monograph on «Bacterial Vaccines».

(e) *Safety Test:* — Four healthy adult guineapigs weighing 300-450 g. not previously treated with any material which will interfere with the test are inoculated subcutaneously, two with 0.2 ml. each and two with 0.5 ml. each of the unglycerinated suspension respectively. Four more guineapigs are injected with 1: 5 dilution of the glycerinated product in the same manner. No untoward reaction should be observed and none of the animals should die of anthrax during the period of observation for seven days.

(f) *Safety and Potency Test in sheep and Goat:* — Spore count of the glycerinated suspension is made after twentyone days from the date of glycerination. Three plates for each of the three dilution 10-5, 10-6 and 10-7 are made.

Eight sheep and eight goats each weighing not less than 18 kg. are injected subcutaneously in the following manner: —

two Sheep: Each subcutaneously with 10 ml. of the stock suspension (for safety).

two Goats: Each subcutaneously with 5 ml. of the stock suspension (for safety).

six Sheep: Each subcutaneously with one million spores suspended in 50 per cent glycerine saline solution.

six Goats: Each subcutaneously with one million spores suspended in 50 per cent glycerine saline solution.

None of these animals should die of anthrax. Twenty one days after vaccination, the animals are challenged with 100 lethal doses of virulent *B. anthracis* spores along with two healthy sheep and two goats as controls.

All the controls should die of anthrax within 72 hours after challenge and at least 66 percent of the vaccinated animals should survive. The animals shall be observed for a minimum of ten days from the date of challenge.

(g) *Viable Count:* — The vaccine when plated on suitable media should show 1.5 million B. anthracis organisms per ml. at the time of bottling, but not less than one million at any time before issue.

5. **Labelling and Storage:** — Should comply with the requirements for "Labelling" and "Storage" as laid down in the general monograph on "Bacterial Vaccines".

6. **Expiry Date:** — The date of expiry of the potency of the vaccine shall be not more than six months from the date of manufacture. The stock suspension can however be stored for two years.

BLACKQUARTER VACCINE

1. **Synonym:** — Blackleg Vaccine or Quarter Evil Vaccine.

2. **Definition:** — Blackquarter Vaccine is a culture of *Clostridium chavvoei* grown in a suitable anaerobic fluid medium and rendered sterile and atoxic by the addition of Solution of Formaldehyde I. P. in such a manner that it retains its *immunising* properties.

3. **Preparation:** — Cultures of **Cl. Chauvoei** are grown in a suitable anaerobic fluid medium and killed by the addition of a suitable concentration of Solution of Formaldehyde I.P. the final product shall be adjusted to PH.7.0.

4. **Standards:** —

(a) *Description:* — It is a yellowish brown liquid containing dead bacteria in suspension.

(b) *Identification:* — It protects susceptible animals against infection with *Cl. chauvoei.*

(c) *Sterility Test:* — Should comply with the test for sterility described in the General monograph on "Bacterial Vaccine".

(d) *Safety and Potency Tests:* — At least six adult healthy guinea-pigs each weighing 300 g to 450 g are injected subcutaneously each with 3 ml. of the product followed a week later by a second injection with the same dose. They should not show any systemic reaction but may show only a minimum of local raction. Fourteen days after the second injection six of the vaccinated guinea-pigs are challenged intramuscularly with 25 viable spores of Cl. Chauvoei equivalent to 5 c.h.d. along with 0.2 ml. of a 5 per cent solution of calcium chloride. Two controls are used. The controls should die of the specific injection and at least 4 of the six vaccinated animals should survive before the product is passed for issue.

5. **Labelling and Storage:** — Should Comply with the requirements of "Labelling" and "Storage" as laid down in the general monograph on "Bacterial Vaccines".

6. **Expiry Date:** — The date of expiry of the potency of the vaccine shall not be more than twenty-four months from the date of manufacture.

BRUCELLA ABORTUS STRAIN 19 VACCINE (LIVING)

1. **Synonym:** — Contagious Abortion Vaccine, (Strain 19) (Living).

2. **Definition:** — Brucella Abortus (Strain 19) Vaccine (Living) is a suspension of a pure smooth living culture of Br. abortus of low virulence in normal saline solution.

3. **Preparation:** — Forty eight to seventy-two hour old growth of *Br. abortus* (Strain 19) on potato agar medium in Toux flasks washed with buffered normal saline solution PH 6.4 and the pure growth from the flasks is pooled together, 0.5 ml. of the pooled product is mixed with 4.5 ml. of normal saline solution at PH 6.4 in graduated centrifuge tube and centrifuged at 3000 r. p. m. for one hour. The percentage of cell deposit is assessed by reading the amount of cell deposit obtained.

The concentrated suspension is then diluted with buffere normal saline solution so that the final product contains 0.72 per cent bacterial cell deposit.

4. **Standard:** —

(a) *Description:* It is an almost white turbid liquid containing live bacteria in suspension.

(b) *Identification:* It consists of Gram-negative bacilli capable of protecting susceptible animals against Brucellosis.

(c) *Sterility Test:* Should comply with the test for sterility described in the general monograph on "Bacterial vaccine".

(d) *Purity Test:* A smear of the finished product is examined microscopically after staining by Gram's method for evidence of any contamination. When grown on suitable media, *Br. abortus* should be obtained in a pure state.

(e) *Safety Test:* Two healthy guinea-pigs each weighing 300 g to 450 g are inoculated sub-cutaneously each with 1.0 ml. of the final product. The guinea-pigs should not show excessive reaction of a toxic nature during the period of observation of ten days.

(f) *Potency Test:* Each of a group of four healthy guinea--pigs, drawn a uniform stock and each weighing 300 g 450 g is injected intramuscularly with 1 ml. of the vaccine, and is challenged nine weeks after vaccination by the intramuscular injection of 1 ml. of a suspension containing 5,000 fully virulent *Br abortus* organims. Each of a group of two unvaccinated guinea-pigs is similarly injected. After a further six weeks, the genineapigs are killed and cultures are made from their spleens. More than half of the vaccinated guinea-pigs contain no demonstrable *Br. abortus* in the spleen; all the controls are infected.

(g) *Viable Count:* The vaccine when plated on suitable media should show between 14,000 million and 18,000 million *Br. abortus* organsims per. ml. At least 80 per cent of the Brucella organisms should be in the smooth phase.

5. **Labelling and storage:** — Should comply with the requirements of "Labelling" and "Storage" as laid down in the general monograph on "Bacterial Vaccines". The liquid vaccine shall be issued fresh as far as possible without allowing any period of storage after manufacture.

6. **Expiry Date:** — The date of expiry of the vaccine shall be not more than five weeks from the date of manufacture.

ENTEROTOXAMEMIA VACCINE

1. **Synonms:** — Clostridium Weichii, Type D, Formal Culture: pulpy Kidney vaccine.

2. **Definition:** — Enterotoxaemia Vaccine is a culture of a highly toxigenic strain of *Clostridium* type D, grown in an anaerobic medium rendered sterile and toxic by the addition of Solution of Formaldehyde I. P. in such a manner that it retains its immunising properties.

3. **Preparation:** — Selected toxigenic strain of **cl. Welchii,** type D, is grown in a liquid medium under conditions which ensure maximum epsilon toxin production. The culture is cheked for purity and toxicity as tested in mice. Solution of Formaldehyde I. is added in suitable concentration and the formolished culture is kept at 37.° C till the production is sterile and non toxic.

4. **Standard:** —

(a) *Description:* It is a yellowish brown liquid containing dead bacteria in suspension.

(b) *Identification:* When injected into susceptible animals it stimulates the production of epsilon antitoxin of cl. Welchii, type D.

(c) *Sterility Test:* Complies with the test for sterility described in the general monograph on 'Bacterial Vaccines'.

(d) *Safety and Potency Tests:* At least eight sheep each weighing not less than 18 kg. or twelve rabbits each weighing 1 kg. to 1.5 kg. are used for testing the safety and potency of each brew of the vaccine. Two sheep receive subcutaneously 10 ml. each and other six sheep receive each 2.5 ml. of the product subcutaneously. The rabbits are given subcutaneously a dose of 5 ml. each. The sheep and rabbits are observed for five days. They should show only a minimum local reaction and no systemic reaction.

The sheep receiving 10 ml. are withdrawn from experiments after five days. Each of the other six sheep is intoculated with a second dose of 2.5 ml. fourteen days after the first injection. The rabbits are inoculated with 5 ml. as a second dose, after one month of the first inoculation. The days after the second inoculation the sera of sheep or rabbits are pooled separately. The pooled serum of each group of animal shall contain in each ml. not less than two international units of Cl. welchii epsilon antitoxin which is determined by testing on mice as follows:

One ml. of the pooled serum is mixed with one ml. of the epsilon toxin of Cl. *welchii* type D, containing 300 mouse-minimum-lethal-doses (mouse m. l. d.) and kept at room temperature for half an hour. At least two mice each weighing not less than 18 g. are each given intravenously 0.2 ml. of the mixture. As control two mice each weighing not less than 18 g. should each receive 0.2 ml. of the toxin containing 300 mouse m.l.d. per ml. diluated with equal volume of normal seline. The control mice should die within 1 to 2 hours while the mice receiving the mixture of serum and toxin should survive for at least two days. Sera containing one International Unit of epsilon antitoxin per ml. will be able to neutralise 150 mouse m.l.d. of epsilon toxin of Cl. welchii, type D.

5. **Labelling and Storage:** — Should comply with the requirements regarding «Labelling» and «Storage» as laid down in the general monograph on «Bacterial Vaccines».

6. **Expiry Date:** — The expiry date of potency of the vaccine shall be not more than twelve months from the date of manufacture.

FOWL CHOLERA VACCINE (POLYVALENT)

1. **Synonym:** — Pasteurella Septica Vaccine (Avisan)

2. **Definition:** — Fowl Cholera Vaccine is a formolised pure broth culture of virulent strains of Pasteurella Septica. (Avian)

3. **Preparation:** — The strains are grown separately in nutrient broth for 48 hours at 37.°C. The pure growth is killed by the addition of a Solution of Formaldehyde I. P. in a suitable concentration. The cultures are then mixed in equal proportions and the final vaccine is bottled in suitable containers.

4. **Standard:** —

(a) *Description:* It is a light yellow liquid containing dead bacteria in suspension.

(b) *Identification:* It protects susceptible birds against *P. aviseptica* infection.

(c) *Sterility test:* Complies with the test for «Sterility» described under the General monograph on «Bacterial Vaccines».

(d) *Safety Test:* — Two healthy young fowls each weighing not less than 400 g. or twelve healthy mice are inoculated subcutaneously each with 1 ml. of the final product. The birds should not show any untoward reaction during the period of observation for seven days.

5. **Labelling and Storage:** — Should comply with the requirements of «Labelling» and «Storage» as laid down in the general monograph on «Bacterial Vaccines».

6. **Expiry Date:** — The date of expiry of potency of the Vaccine shall not more than six months from the date of manufacture.

HEMORRHAGIC SEPTICAEMIA ADJUVANT VACCINE

1. **Synonym:** — Pasteurella Septica Adjuvant Vaccine.

2. **Definition:** — The vaccine is a homogenous suspension of formolished agar-washed Pasteurella septica with liquid paraffin and lanolin.

3. **Preparation:** — Pure growth of a highly antigenic strain of **P. Septica** in phase 1 grown on nutrient agar medium containing 0.5 per cent yeast extract is washed with 0.5 per cent formal saline. The pooled suspension is diluted with formal saline to contain approximately 2100 million *P. Septica* organisms per ml. The safety test of this adjusted suspension is conducted on four white mice each weighing not less than 18 g. and observed for three days before it is mixed with liquid paraffin and lanolin in suitable proportion.

The mixture is blended until a homogenous emulsion is obtained which is filled in suitable containers.

4. **Standard:** —

(a) *Description:* It is a white thick oily liquid containing dead bacteria in suspension.

(b) *Identification:* It protects susceptible animals against infection with P. Septica.

(c) *Sterility Test:* It compies with the test for «Sterility» described in the general monograph on «Bacterial Vaccines».

(d) *Safety Test:* Six white mice each weighing not less than 18 g. are inoculated inraperitoneally each with 0.5 ml. of the vaccine. None of the mice should die of pasteurellosis during the observation period for seven days.

(e) *Potency Test:* Three susceptible calves in good condition between the ages of nine months to three years are injected intramuscularly, each with 2 ml. of the vaccine, in the case of animals weighing upto 140 kg. and 3 ml. for heavier ones.

Three weeks later these animals along with two healthy animals of the same type and species are challenged subcutaneously with 18 hours old broth culture of *P. septica* equivalent to at least 50 million mouse minimum infective dose. Both the controls should die of pasteurellosis and at least two out of the three protected animals should survive the challenge dose for a period of seven days.

5. **Labelling and Storage:** — Should comply with the requirements for «Labelling» and «Storage» as laid down in the general monograph on «Bacterial Vaccines».

6. **Expiry Date:** — The date of expiry of potency of the vaccine shall not more than twelve months from the date of manufacture.

HAEMORRHAGIC SEPTICAEMIA VACCINE (BROTH)

1. **Synonym:** — Pasteurella Septica Vaccine (Broth).

2. **Definitions:** — Haemorrhagic Septiceemia Vaccine is formolised culture of a virulent strain of Pasteurella septica in nutrient broth.

3. **Preparation:** — P. Septica culture is grown in nutrient broth at 37°C. The pure growth is killed by the addition of a solution of Fermaldehyde I. P. in a suitable concentration.

4. **Standard:** —

(a) *Description:* It is pale yellow liquid containing dead bacteria in suspension.

(b) *Identification:* It protects susceptible animals against infection with *P. Septica.*

(c) *Sterility Test:* Complies with the test for «Sterility» described under the general monograph on «Bacterial Vaccines».

(d) *Safety Test:* Four healthy rabbits each weighing 1 kg. to 1.5 kg. are inoculated subcutaneously each with 5 ml. of the product. There should be no untoward reaction during the period of observation for seven days. Alternately two rabbits and six mice may be employed. The dose for mice will be 0.5 ml.

5. **Labelling and Storage:** — Should comply with the requirements of «Labelling» and «Storage» as laid down in the general monograph on «Bacterial Vaccines».

6. **Expiry Date:** — The date of expiry of potency of the vaccine shall not more than six months from the date of manufacture.

SALMONELIA ABORTUS EQUI VACCINE

1. **Synonym:** — Equine Abortion Vaccine.

2. **Definition:** — Equine Abortion Vaccine is a mixture of equal parts of pure formolised cultures of smooth laboratory strains of Salmonella abortus equi.

3. **Preparation:** — The strains are grown separately on plain agar in Rouz flasks, for 24-28 hours at 37°C. The pure growth is washed with normal saline solution and the washings are pooled together. The suspension is standardised to contain approximately 600 million *Sal. abortus equi* organisms per ml. using normal saline solution as diluent. The culture is killed by the addition of sufficient quantity of solution of Formaldehyde I. P. in a suitable concentration and the product is kept at 37°C for seven days. Potassium alum is added to give a final concentration of 1 per cent.

4. **Standard:** —

(a) *Description:* It is an opalescent liquid containing dead bacteria in suspension.

(b) *Identification:* It protects susceptible animals against infection with *Sal. abortus equi.*

(c) *Sterility Test:* S Complies with the tests for sterility described in the general monograph on «Bacterial Vaccines».

(d) *Safety Test:* Six white mice each weighing not less than 18 g. are inoculated intraperitoneally each with 0.5 ml. of the product. None of the mice should die of salmonellosis. The mice are observed for ninety-six hours.

5. **Labelling and Storage:** — Should comply with the requirements for «Labelling» and «Storage» as laid down in the general monograph on «Bacterial Vaccines».

6. **Expiry Date:** — The date of expiry of potency of the vaccine shall be not more than six months from the date of manufacture.

STREPTOCOCCUS EQUI VACCINE

1. **Synonym:** Strangles Vaccine.

2. **Definition.:** — Streptococus equi vaccine is a phenolised culture of a number of different isolates of *Streptococcus equi* in glucose serum broth.

3. **Preparation:** — Equal proportions of forty-eight hours old pure cultures of different isolates of *Str. equi* in serum glucose broth are mixed together. The suspension is centrifuged and the deposit is washed with normal saline solution after removing the supernatant. The washed cells are suspended in normal saline and heated in a water bath 65°C for two hours. Phenol and normal saline are added to give a final concentration 1200 million Str. equi organisms per ml. and 0.5 per cent of phenol in the vaccine.

4. **Standard:** —

(a) *Description:* It is a slightly opalescent liquid containing dead bacyeria in suspension.

(b) *Identification:* It protects susceptible animals against infection with *Str. Equi.*

(c) *Sterility Test:* Complies with the test for «Sterility» described in the general monograph on «Bacterial Vaccine». The nutrient broth being replaced by glucose broth.

(d) *Safety Test:* Two ponies and two rabbits each weighing not less than 1 kg. are inoculated each with 10 ml. and 2 ml. respectively of the final product. The animals should not show any untoward reaction during the period of observation of seven days.

5. **Labelling and Storage:** — Should comply with the requirement for «Labelling» and «Storage» as laid down in the general monograph on «Bacterial Vaccines».

6. **Expiry Date:** — The date of expiry of potency of the Vaccine shall be not more than six months from the date of manufacture.

OLD ADJUVANT VACCINE AGAINST PASTEURELLOSIS IN SHEEP AND GOATS

1. **Synonym:** — Pasteurella Septica Adjuvant Vaccine for ovines and Caprines.

2. **Definition:** — The vaccine is a homogenous suspension of formalised agarwashed Pasturella septica of ovine origin with liquid paraffin and lanolin.

3. **Preparation:** — Pure growth of highly antigenic strains (R_1, R_2, R_4) in phase I grown separately on neutrient agar-medium containing 0.5 per cent yeast extract is washed with 0.5 per cent Normal saline. Equal quantities of the suspension of three strains diluted with Normal sline to contain approximately 2100 million organisms per ml. is pooled together. The safety test of this adjusted polled suspension is conducted in four white mice each weighing not less than 18 g. and observed for three days before it is mixed with liquid paraffin and lanolin in suitable proportion.

The mixture is blended until a homogenous emulsion is obtained which is filled in suitable containers.

4. **Standard:** —

(a) *Description:* It is a white thick oily liquid containing dead bacteria in suspension.

(b) *Identification:* It protects susceptible animals against infection with P. Septica.

(c) *Sterility test:* Complies with the test for «sterility» described in the general monograph on «Bacterial vaccines».

(d) *Safety test:* Six white mice each weighing not less than 18 g. are inoculated intra-peritoneally each with 0.5 ml. of the vaccine. None of the mice should die of Pasteurellosis during the observation period of seven days.

The vaccine is also inoculated into six sheep and six goats in a dose of 3 ml. each intramuscularly and are observed for a period of seven days. During this period none should die of Pasteurellosis.

(e) *Potency Test:* — Not being done at present.

5. **Labelling and Storage:** — Should comply with the requirements regarding «Labelling and storage» as laid down in the General monograph on «Bacterial Vaccines».

6. **Expiry Date:** — The expiry date of Potency of the Vaccine shall be not more than twelve months from the date of manufacture.

(B) *Provisions applicable to the production of Viral Vaccines.*

1. **Definition:**

(i) This part of the Schedule applies to viral vaccines live or inactivated made from any virus pathogenic to domestic animals and poultry and made from other modified viruses which have any antigenic value.

(ii) For the purpose of this part of the Schedule, a virus vaccine means a sterile suspension or a freeze dried powder

containing the modified living or inactivated virus particles, which, in its original unaltered stage, causes disease from which the vaccine derives its name and which has been prepared from the blood or tissues of a suitable host in which it has been grown in *vivo* or from tissue culture.

2. **Staff of Establishment:** — The establishment in which viral Vaccines are prepared, must be under the direction and control of an expert in bacteriology with specialised training in virology and sufficient experience in the production of viral vaccines, and he shall be assisted by a staff adequate for carrying out the tests required during the preparation and standardisation of the vaccines.

3. **Proper Name:** — The proper name of any viral vaccine shall be the name of the disease which is caused by the particular virus from which the vaccine is produced followed by the word «Vaccine» unless the Schedule otherwise provides, or if there is no special provision in the Schedule such other name as is approved by the licensing authority; Provided that in the case of the undermentioned preparations the proper name of the vaccine shall be as follows: —

i) Fowl Pox Vaccine, Chick Embryo Virus (Living).
ii) Fowl Pox Vaccine, Pigeon Pox Virus (Living).
iii) Horse Sickness Vaccine (Living).
iv) Ranikhet Disease Vaccine (Living).
v) Ranikhet Disease vaccine F Strain (Living).
vi) Randerpest Goad Adapted Tissue Vaccine (Living).
vii) Rainderpest Lapinised Vaccine (Living).
viii) Rinderpest Lapinised Avianised Vaccine (Living).
ix) Sheep and Goat Pox vaccine (living).
x) Swine fever vaccine (crystal voilet).
xi) Swine fever vaccine lapinised (living).

4. **Records:** — Viruses used in the preparation of vaccine must before being used for preparing a batch be thoroughly tested for purity, safety, sterility and antigenicity by the generally accepted tests applicable to the particular virus. The permanent records which the licensee is required to keep shall include a record of the origin, properties and characteristics of the seed virus from which the vaccines are made.

5. **Tests:** — Viral vaccine shall be tested for sterility, safety and potency on suitable test animals and for viability in the case of live vaccines.

(a) *Sterility Test:* All vaccines shall be tested for sterility in accordance with rules 115 to 119. If the vaccine contains added bactericides or bacteriostatic, a quantity of medium sufficient to render the growth inhibitor ineffective is added to the sample or a suitable substance is added in a concentration sufficient to render the growth inhibitor ineffective but not it self to inhibit the growth of microorganism.

(b) *Safety Test:* Suitable laboratory animals or large animals or birds may be employed to test the vaccine for safety. Details of safety test are given in the individual monograph.

(c) *Potency Test:* All virus vaccines for which potency test has been prescribed shall be tested for potency and only those which pass the potency test shall be issued. Details of the potency test are given in the individual monograph.

6. **Storage:** — Live viral vaccines shall be stored, protected from light at sub-zero temperature as required. Other viral vaccines shall be stored at 2°C to 4°C but shall not be frozen.

7. **Condition of housing of animals.**

(i) The animals used in the production of vaccine must be housed in hygienic conditions in premises satisfactory for this purpose.

(ii) Only healthy animals may be used in the production of vaccine. Each animal intended to be used as a source of vaccine must, before being passed for the production of vaccine be subjected to a period of observation in quarantine for at least seven days. During the period of Quarantine the animal must remain free from any sign of desease and must be well kept.

8. **Labelling:** — The provisions of «Labelling» as laid down for Bacterial Vaccines shall also apply to Viral Vaccines. The following additional information shall also be included on the lable of the outside container.

(i) The name and percentage of bacteriostatic agent contained in the vaccine.

(ii) If the vaccine as issued for sale contains any substance other than the difuent, the nature and strength of such substance.

9. **Date of Manufacture:** — For the purpose of this part of the Schedule, the date of manufacture shall be what is given unless otherwise stated in the individual monograph, as defined in sub-clause (b) of sub-rule (3) of rule 109.

FOWL POX VACCINE, CHIK-EMBRYO VIRUS (LIVING)

1. **Synonym:** — Egg-adapted Fowl Pox Vaccine (Living).

2. **Definition:** — Fowl-pox vaccine, Chick-Embryo Virus (Living) is a suspension of a modified living virus (e.g. Mukteswar strain) prepared from the cherioallantoic membran. (CAM) of the infected embryo and is either freeze dried or is issued as glycerinated liquid vaccine.

3. **Preparation:** — Active chick-embryos obtained from Salmonella pullorum free flock, are used. Twelve to thirteen day old embryos are injected membrene (stock seed virus). The suspension of the stock seed virus is dropped on the CAM. After an incubation at 37°C for a suitable period membrances showing discrete or confluent lessions (pocks) are harvested. These are homogenised with adequate quantity of antibiotics (penicillin and streptomycin) ampouled in 0.5 ml. quantities and freeze dried.

4. **Standard:** —

(a) *Description:* Light mauve coloure scales.

(b) *Identification:* When reconstituted vaccine is applied to scarified area of the skin of a fowl it produces characteristic lesions of fowl pox. This product should afford protections gainst fowl pox.

(c) *Moisture Content:* Moisture Content in the finished product should not exceed 1.0 per cent.

(d) *Safety Test:* For testing each batch of fowl pox vaccine twelve healthy cockerols or other suitable young chicken each weighing not less than 400 g. from the same source are taken. This group of twelve birds is immunized at least twenty one days previous to the test, with fowl pox vaccine. The vaccine under test is reconstituted in 5 ml. of 50 per cent glycerine saline and administered to fowls as follows:

Three of the test birds are injected subcutaneously with 0.8 ml. or 10 items the field doses of the vaccine under test. This group serves to indicates whether the product is free from other viruses and bacteria causing septicaemia or not.

Three of the test birds are injected intratrecheally with 0.3 ml. or 10 items the field dose of vaccine under test. This group serves to indicate whether the product is free from the virus of infectious laryngotracheitis and simllar diseases.

Three of the test birds are injected intranasally with 0.2 ml. of the vaccine under test. This group serves to indicate whether the product is free from the virus of Coryzai and similar diseases.

Three of the test birds are injected intranasally with 0.2 ml, of the vaccine under test. This group serves to indicate whether the product is free from the virus of Coryzai and similar disease.

The three remaining birds serve as controls. They are isolated and kept under observation for twenty-one days. The birds that succumb during the period of twenty-one days are subjected to a careful postmortem examination. The product is withheld from issue until the vaccine and the test birds are shown to be free from the causative agents of any extrancous disease.

(e) *Sterility test:* — Complies with the tests for sterility. A described under the general monograph on «viral vaccines».

(f) *Potency Test:* — For testing of potency three unsceptible birds each weighing not less than 400 g. are vaccinated using the field dose by the stick method and examined for «takes». Three weeks after vaccination these birds along with two unvaccinated controls are exposed to challenged virus and observed for fourteen days. The vaccinated birds should not manifest any reaction, while the controls should show active «takes».

5. **Labelling:** — Should comply with the requirement for «Labelling» as laid down in the general monograph on 'Viral Vaccines'.

6. **Storage and Expiry date:** — Freeze dried vaccine shall be expected to retain its potency for periods at temperatures as specified below: —

-15°C to -20°C — Twenty four months.
20°C to 40°C — Twelve months.
Room temperature — upto one month.

The liquid vaccine shall be expected to retain its potency for periods and temperatures as specified below: —

2°C to 4°C — Six months.
Room temperature — seven days.

FOWL-POX VACCINE PIGEON POX VIRUS (LIVING)

1. **Synonym:** — Fowl-Pox Vaccine (pigeon pox scab).

2. **Definition:** — Fowl-pox vaccine, pigeon-pox virus (living) consists of pigeon pox virus in scabs collected from artificially infected pigeons and dried.

3. **Preparation:** — Healthy pigeons are scarified on the legs and Breast, with a suitable dilution of the suspension of pigeon-pox virus. The pigeons reacting satisfactorily and showing good takes are selected and the superficial skin layer scraped by means of sharp scalpel. The material so collected is freed from feathers, homogenised and dried or freeze dried. The dried pulp is powdered, sieved and ampouled in Powdered, sieved and ampouled in 0.3 quantities an sealed.

4. **Standard:** —

(a) *Description:* — Light cream coloured powder.

(b) *Identification:* — When applied to feather follicles by vigorous rubbing, it produces mild reaction in fowls. The product should afford protection to fowls upto six weeks against fowl pox.

(c) *Safety Test:* — For testing a batch of vaccine, twelve healthy cockerels, or other suitable young chicken from the same source are made available at the same time. This group of twelve birds is immunised at least twenty-one days previous to the test with fowl pox vaccine. The vaccine under test is reconstituted in 10 ml. of 50 per cent glycerine saline and administered to fowls as follows: —

Three of the test birds are injected subcutaneously with 0.3 ml. or 10 items the field dose of the vaccine to be tested. This group serves to ind cate whether the product is free from organisms of septicaemia diseases.

Three of the test birds are injected intratrecheally with 0.3 ml. or ten times the field dose of the vaccine to be tested. This group serves to indicate whether the product is free from the virus of infectious laryngotrachoitis and similar diseases.

Three of the test birds are injected intranasally with 0.2 ml. of the vaccine to be tested. This group serves to indicate whether the product is free from virus of Goryza and similar diseases.

The three remaining birds serve as controls. All the birds under test are isolated and held under observation for twenty-one days. All those that succumb are subjected to careful postmortem examination. The product is withheld from issue until the vaccine and test birds are shown to be free from the causative agents of any extraneous disease.

(d) *Sterility Test:* Complies with the tests for sterility described, under the general monograph on «Viral Vaccines».

(e) *Potency Test:* For testing the potency of a batch of vaccines three susceptible birds each weighing not less than 400 g. are vaccinated using the field dose by the follicular method and examined for «takes». Three weeks after vaccination these birds and two healthy suscepitable controls are exposed to challenge virus and are observed for fourteen days. The vaccinated birds shall manifest no reaction, while the controls must have active «takes».

5. **Labelling and Storage:** — Should comply with the requirements of «labelling» as laid down in the general monograph on 'Viral Vaccines'.

6. **Storage and Expiry date:** — The vaccine shall be expected to retain its potency for periods at temperatures as specified below:

15°C to — 20°C — two years
2°C to 4°C — twelve months

FOWL POX VACCINE PIGEON POX CHICK EMBROYOS VIRUS (LIVING)

1. **Synonym:** — Chick embryo adapted gieon pix vaccine (Living).

2. **Definition:** — Fowl pox vaccine (Pigeon Pix virus) chick embryo adapted virus (living) is a suspension of a modified living virus prepared from the choncaianuc membranes of the infected embroyos and is freeze dried.

3. **Preparation:** — Active chick embryos obtained from Salmonella Pullorum free stock are used. Twelve to thirteen days old embryos are injected with a suitable dilution of the suspension of the infected membrane (stock seed virus) of chick embryo adapted pigeon pox virus. The suspension of the stock seed virus is dropped on the membrane. The inoculated eggs are incubated at 37°C for four days. One of the fourth day embryos that are living, are removed to a refrigerator for chilling for about one hour. Membranes showing discrete lesions (Pocks) are harvested. These are homogenised with adequate quantities of antibiotics, ampouled in 0.5 ml. quantities and freeze dried.

4. **Standard:**

(a) *Description:* — Light mauve colured scales.

(b) Indentification: — When reconstituted vaccine is applied to scarified area of the skin of a fowl, it produces characteristic lesions of Fowl Pox. This product should afford protection against pox.

(c) *Moisture content:* — Moisture content in the finished product should not exceed 1.0 per cent.

(d) *Safety test:* — For testing each batch of chicks aged four to six weeks from the same source are taken. This groups of twelve birds is immunized at least twenty-one days previous to the last, with fowl, pox vaccine. The vaccine under test is reconstituted in 3 ml. of normal saline solution and administered as under: —

Three of the test chicks are injected subcutaneously with 0.3 ml. or 10 items the field dose of the vaccine under test. This group serves to indicate whether the product is free from other viruses and bacteria causing of septicaemia or not.

Three of the test chicks are injected intra traceableally with 0.3 ml. or tem items the field dose. This group serves to indicate whether the product is free from the viruses of infections laryngeotrachiti and similar diseases.

Three of the test chick are injected with 0.2 ml.1/N of the vaccine under test. This group serves to indicate the product is free from the virus of coryza and similar diseases.

For remaining three chicks serve as controls. They are isolated and kept under observation for twenty one days. The birds that succumb during the period of observation are subjected to careful post-mortem examination. The product is withheld from issue until the vaccine and the test birds are shown to be free from the causative agents of any extraneous disease.

In addition to the above, similar groups of pigeons aged six to nine months old are also injected in a similar way to eliminate psittacosis.

(e) *Sterility test:* — Should comply with the tests for sterility described under the general monograph on 'Viral Vaccine'.

(f) *Potency test:* — For testing potency of a batch of vaccine, three susceptible chicks of three to four weeks of age are vaccinated by feather fothcle method. (A few fothicles on one leg are injected) and these are examined for 'takes'.

Three weeks after vaccination these chicks along with two unvaccinated chiks are exposed to challenge virus (virulent fowl pox virus) and observed for fourteen days. The vaccinated chicks should not manifest any reaction while controls should shown active 'takes'.

5. **Labelling:** — Should comply with the requirements for 'Labelling' as laid down in the general monograph on 'Viral Vaccines'.

6. **Storage:** — The freeze dried product is expected to retain its potency for periods and temperature as specified below: —

-15°C to -20°C two years.
2°C to 4°C twelve months.
Room temperature upto one month.

SHEEP POX VACCINE (LIVING)

1. **Synonym:** — Sheep Pix vaccine; Goat pox vaccine.

2. **Definition:** — Sheep pox vaccine consists of sheep pox virus collected from sheep artificially infected with sheep pox virus and freeze dried.

3. **Preparation:** — Healthy yearling sheep are infected artificially by subcutaneous infection on the undersurface of the previously shaved abdomen with 200-300 cc. of the freeze dried sheep pox virus (seed material) diluted in 1:1Normal saline solution on the sixth or seventh day after injection cedematous swelling develops in the injected area with thermal reaction. The sheep which develop good swelling are slaughtered and the gelatinous material present under the skin in the infected are is collected under sterile conditions. This material is mixed with 2 parts by volume of sterile peptone broth of PH 7.2 and homogenised. The homogenised suspension is filtered, ampouled in 0.5 ml. quantities and freeze dried.

4. **Standard:**

(a) *Description:* — While scales.

(b) *Identification:* — Reconstituted vaccine when applied over the scarified area of the skin of the abdominal region of sheep will produce characteristic local lesion of.

(c) *Moisture content:* — The moisture content should not exceed 1.0 per cent.

(d) *Safety test:* — Two rabbits each weighing not less than 1 kg. are injected subcutaneously each with 1 ml. of 1:100 dilution of the vaccine in normal saline solution. These animals are observed for fourteen days. The animals should ramain normal.

(e) *Sterility Test:* — Complies with the tests for sterility described under the general monograph on 'Viral Vaccines'

(g) *Potency Test:* — Four yearling sheep are vaccinated on the inder surface of the ear by scafification method. The contents of one ampoule of F.D. Sheep Pox vaccine are constituted in 10 c.c. of 50% glycerine saline solution, characteristic takes develop in the scarified are with ulceration and scab formation. Three weeks later these and two more suceptible sheep (Controls) are challenged by scarifiying with a suspension of the previous brew of the vaccine of the undersurface of the abdomen. The controls should develop typcal lesions of pox and the vaccinated should remain normal.

5. **Labelling:** — Should comply with the requirements of 'labelling' as laid down in the general monograph on Viral Vaccine.

6. **Storage and expiry date:** — The vaccine is expected to retain potency for period and temperature as specified below: —

-15°C to -20°C two years.
2°C to 4°C three months.

HORSE SICKNESS VACCINE (LIVING)

1. **Synonym:** — African Horse Sickness Vaccine; Mouse adapted Polyvalent Horse Sickness Vaccine (Living).

2. **Definition:** — Horse sickness vaccine is a suspension of live mouse adapted strains of Horse Sickness Virus (onderstepoort) prepared from the brains of infected mice and is freeze dried.

3. **Preparation:** — Thirty to thirty-five day old white mice are infected intracerabrally with 0.05 ml. of a suitable dilution of the seed virus (6 or 7 types, as the case may be). Groups of large numbers of mice are injected separately with each type of the virus and are housed at 27°C to 32°C. A majority of these become paralytic on the third and fourth day when they are sacrificed and their brains collected and stored at -15°C to -20°C till the day of processing. For preparing the polyvalent vaccine, equal number of brains collected from mice infected with different types of the virus are homogenised with 5-10 times its volume of sterile lactose buffer medium (PH 7.2) containing antibiotics. The suspension is centrifuged at 1500 r. p. m. for five minutes. The supernatant liquid is distributed in ampoules in suitable quantities and freeze dried.

*Room temperature fifteen days.

4. **Standard:**

(a) *Description:* — White scaly material.

(b) *Identification:* — This product affords protection to horse against horse sickness.

(c) *Safety Test:* — Four healthy mice thirty to thirty-five days old are injected intraperitoneally with 0.2 ml. of 10-1 dilution of the vaccine and kept under observation for ten days. All the mice should remain normal throughout the period of observation.

(d) *Sterility test:* — Should comply with the test for sterility described under the general monograph on 'Viral Vaccines'.

(e) *Viability Test:* — Each batch of vaccine is titrated in ten fold dilutions using four mice of thirty to thirty-five days old for each dilution. Each mouse is injected intracerebrally with 0.05 ml. and kept under observation for ten days. Mortality and survival ratios are noted and LD50 is determined. The minimum acceptable titre is 10-4 LD50 per 0.05 ml.

5. **Labelling:** — Should comply with the requirements of 'Labelling' as laid down in the general monograph in 'Viral Vaccine'.

6. **Storage:** — The vaccine may be expected to retain its potency for twelve months if stored at 15°C to -20°C. and about six months if stored in refrigerator at 2°C to 4°C.

RABIES VACCINE (INACTIVATED)

1. **Synonym:** — Antirabic Vaccine (Inactivated).

2. **Definition:** — Rabies vaccine is a suspension of the brain tissue of animals, that have been infected with a suitable strain of rabies fixed virus, inactivated with phenol or some other suitable agent.

3. The following particulars relating to this vaccine are the same as those relating to Antirabic Vaccine described in Part D of Schedule F to these rules, namely: —

i. Strain of fixed Rabies Virus to be used;
ii. Staff of Establishment;
iii. Condition and housing of animals;
iv. Precaution to be observed in preparation;
v. Records;
vi. Issue.

4. **Preparation:** — Healthy sheep or any other suitable species of animal are inoculated subburelly or intracerebrally with an appropriate dose of suspension of a suitable strain of rabbit brain passaged rabies fixed virus. The sheep or animals which get paralysed from the sixth day onwards after the inoculation are sacrificed and their brains collected aseptically. Brain tissue is weighed individually and a suspension of suitable concentration of brain tissue prepared in buffered saline is strained through gauze. The suspension treated with phenol or some other suitable inactivating agent is incubated for an appropriate period.

5. **Standard:**

(a) *Description:* — A grey to pale yellow opalescent suspension.

(b) *Identification:* — Appropriate doses protect mice against subsequent intracerebral inoculation with suitable strain of fixed rabies virus.

(c) *Safety test:* — Not less than five mice each weighing at least 18 gm., are inoculated inrecerebrally with not less than 0.03 ml. of the suitable diluted vaccine. None of the animals should show symptoms of rabies for die of the disease during period of observation of three weeks.

(d) *Sterility Test:* — Should comply with the test for sterility described under the general monograph on 'Viral Vaccine'.

6. **Labelling:** — Should comply with the requirements of labelling as laid down in the general monograph on 'viral vaccines'. In addition the label on the container shall indicate the percentage of brain tissue present in the vaccine.

7. **Storage:** — The vaccine may be expected to retain its potency for about six months if stored in refrigerator at 2°C to 4°C.

RABIES VACCINE (LIVING)

1. **Definition:** — Rabies vaccine (living) is a freeze dried suspension of chick-embryo tissue infected with a suitable attenuated strain of rabies virus.

2. **Preparation:** — It may be prepared by the following method. Seed virus consisting of a suspension of the Flury or other suitable strain of chick adapted virus that has been maintained by passage in chick embryos is injected into the yolksacs of fertile eggs incubated for a suitable period. After incubation for a further ten days, the embryos are harvested and ground in water for injection to give 33 per cent suspension. The suspension is centrifuged to remove coarse particles and the supernatant fluid is distributed into ampoules in 3 millitre quantities, and freeze-dried. The vaccine is reconstituted immediately before use by adding 3 millilitres of water for injection to the contents of an ampoule.

3. **Standard:** — It complies with the requirements of general standard of viral vaccines for abnormal toxicity, sterility, and labelling, with the following additions.

(a) *Description:* — Dry honey-coloured flakes or powder, readily dispersible in water.

(b) *Identification:* — It protects guinea pig against a subsequent inoculation of rabies street virus. It is distinguished from the inactivated Rabies vaccine by its ability to produce rabies encephalitic on intracerabral injection into mice.

(c) *Safety:* — The guinea-pigs used in the test for potency should not show any marked local or systemic reaction during the three weeks following injection with the vaccine.

(d) *Sterility Test:* — Complies with the tests for sterility described under the general monograph on «Viral Vaccines».

(e) *Potency:* — The contents of an ampoule are dispersed in water for injection to give a 5 per cent suspension and not fewer than twenty guinea pigs, drawn from a uniform stock and each weighing 350 g. to 500 g., are each injected intramuscularly with 0.25 ml. of this suspension. Three weeks later, these guinea pigs and an equal number of similar unvaccinated control guinea-pigs are each inoculated with 0.1 ml. of a suitable dilution of cenine salivary gland suspension of street virus which is maintained as a 20 per cent suspension at 70°C or lower. The guinea pigs and observed for thirty days; nos less than 80 per cent of the control guinea pigs die of rabies and not less than 70 per cent, of the vaccinated guinea pigs are protected.

4. **Storage:** — Freeze-dried vaccine should be stored at refrigeration temperatures of 2°C to 4°C.

5. **Labelling:** — The life of the vaccine at room temperature and at refrigeration temperature should be stated on the label.

6(a) **Action and uses:** — Rabies vaccine (living) is used for the prophylactic inoculation of dogs against rabies; one injection should provoke a serviceable immunity lasting for at least a year. The vaccine has been used to a limited extent on cattle.

6(b) **Dose:** — By intramuscular injection: Dogs, the contents of one ampoule reconstituted in 3 ml. of water for injection; cattle five times the dog dose.

RANIKHET DISEASE VACCINE (LIVING)

1. **Synonym:** — New castle Disease Vaccine (Living); pheumoenteritis Vaccine (Living).

2. **Definition:** — Ranikhet Disease vaccine is a suspension of a modified living virus e. g. (Mukteswar strain) prepared from infected embryos and fluids and is freeze dried.

3. **Preparation:** — Good fertile eggs obtained from Calmonella pullorum free flock are incubated in an egg incubator. Ten days old vigorous embryos are infected with 0.1 ml of a suitable dilution of a suspension of the virus. Inoculation is done in the allantoiccavity. Embryos are incubated at suitable temperature, eggs showing dead embryos twenty-four hours after incubation are discarbed. After forty-eight hours incubation, the eggs are candled and those showing dead embryos are chilled for a suitable period of time while embryos alive beyond fortyeight hours are discarded. The fluids and embryos are then collected and spot haemogglutination carried out. The material is homogenised in a blended and ampouled in a aliquots of 0.5 ml. quantities freeze dried.

4. **Standard:**

(a) *Description.* — Light brown scales.

(b) *Identification.* — This product affords protection to fowls against Ranikhet Disease.

(c) *Safety Test:* — For testing each batch of freeze dried Ranikhet Disease Vaccine, twelve healthy young chickens, all from the same source each weighing not less than 400 g. are taken and immunised against Ranikhet Disease. Fourteen days later, these birds, are tested as follows with the contents of one ampoule suspended in 100 ml. of normal saline.

Three of the test birds are injected subcutaneously with 0.1 ml. equivalent to ten times the field dose of the vaccine to be tested. This group serves to indicate whether the product is free from viruses or organisms of septicaemia disease.

Three of the test birds are injected in ratracheally with 0.1 ml. equivalent to ten times the field dose of the vaccine to be tested. This group serves to indicate whether the product is free from the virus of infections larnogotracheitis. Coryza and similar diseases.

The three ramaining birds serve as controls.

All the treated birds and controls are observed daily for fourteen days. All the test birds that succumb are subjected to careful postmortem examination. The product is not issued until the birds under test are shown to be free from the causative agents of any extraneous diseases.

(e) *Sterility Test:* — Should comply with the test for sterility described in the general monograph on 'Viral Vaccine'.

(f) *Potency Test:* — Four susceptible birds eight to twelve weeks old and each weighing not less than 400 g. are vaccinated by injecting subcutaneously 1 ml. of a 10-6 dilution of the product. Two weeks after vaccination these birds and four non-protected birds are challenged by injecting subcutaneously each with 1.0 ml. of a 1:100 dilution of virulent virus (liver and spleen suspension) or 1.0 ml. of a 1:100 dilution of fluid from the embryo infected with virulent Ranikhet Disease Virus. The non-protected birds should show symptoms of Ranikhet Disease and die and all the protected birds should remain normal during an observation period of fourteen days.

5. **Labelling:** — Should comply with the requirements of 'Labelling' as laid down in the general monograph on 'Viral Vaccines'.

6. **Storage:** — The vaccine when stored at — 15°C to 20°C may be expected to retain the potency for about one year and about three months if stored in a refrigerator at 2°C to 4°C. The product should not be used if stored for more than ten days outside the refrigerator.

RANIKHET DISEASE VACCINE F STRAIN (LIVING)

1. **Synonyms:** — New castle disease vaccine F strain (Living).

2. **Definition:** — Ranikhet disease vaccine F. strain is a suspension of a naturally modified living virus (F stain) prepared from the infected embryos, devoid of beaks and eyes and fluids in freeze state.

3. **Preparation:** — Good fertile eggs obtained from salmonella Pullorum free flock are incubated in an egg incubator. Eight days old vigorous embryos are infected with 0.1 ml. of 1:100 suspension of Ranikhet Disease vaccine F strain virus. Inoculation is done via the allantoic cavity. Embryos are incubated at 37°C. Eggs are candled every day upto four days and the dead ones are discarded. Final candling of the embryos is carried out on the fourth day and only the living mones are chilled in a refrigerator for one hour. The fluids embryos are collected separately. The fluids are tested for spot haemagglutination and sterility test is carried. The beaks and eyes balls of the embryos are removed. The materials are homogenised with adequate quantities of antibiotics in a cool warning blended and ampouled in aliquots of 0.5 ml. quantity and Freeze dried.

4. **Standard:**

(a) *Description:* — Light brown scales.

(b) *Identification:* — This product affords protection to baby chicks against Ranikhet disease.

(c) *Moisture content:* — The moisture content should not exceed 0-1 percent.

(d) *Potency test:* — For testing each batch of the vaccine twelve one-day-old chicks are given two drops 1/N of the field dose of the vaccine (5 ampoules selected at random may be reconstituted in 50 ml.) of cold normal saline solution. These are observed for fourteen days and the vaccinated chicks should remain normal throughout the period of observation. This serves the safety test also.

On the fourteen days the vaccinated chicks are challenged two drops with 1:50 virulent Ranikhet Disease virus alongwith 8 control chicks. Four of the controls received two drops 1/N of the virulent virus while the rest of the four receive 0.5 ml. of the virulent virus. The control chicks should succumb to the challenge virus showing symptoms of Ranikhet Disease while the protected chiks should remain normal throughout the observation period of fourteen days.

(e) *Sterility test:* — Should comply with the tests for sterility described in the general monograph on viral vaccines.

5. **Labelling:** — Should comply with the requirements of «Labelling» as laid down in the general monograph on «Viral Vaccines».

6. **Storage:** — The vaccine when stored at — 15 to 20°C may be expected to retain the potency for about one year and about three months if stored in a refrigerator at 2 to 4°C. When removed from the refrigerator, a the product should not be used later than ten days.

RINDERPEST GOAT ADAPTED TISSUE VACCINE (LIVING)

1. **Synonym:** — Goat-adapted Cattle Plague Vaccine; Goat Tissue Vaccine (Living).

2. **Definition:** — Rinderpest Goat-adapted Tissue Vaccine is the homogenised freeze dried prepartion of spleen pulp of goats artificially infected with the suitable strain of rinder-pest virus.

3. **Preparation:** — Healthy suspetible goats are quarantined for a period of ten days. After this period a batch of selected goats are injected subcutaneously with 2 ml. of a suitable dilution of the suspension of the seed virus. The donor goats are sacrified after a suitable period when the titre of the virus in the animal body is expected to be maximum; usually four days and the spleen from animals free from any pathological change or signs are collected under sterile conditions. Smear from each spleen is examined microscopically to exclude spleens which are contaminated from the production batch.

The spleen is freed from fat and fascia and is blended into a smooth pulp is a grinder. The pulp is spread on a shallow dish of glass or stainless steel and is freeze dried.

The freeze dried pulp is then ground into a fine powder and sieved. The powder is ampouled in 0.25 g. or 0.125 g. quantities and freeze dried.

4. **Standard:**

(a) *Description:* — Dark brown or chocolate coloured scales or powder.

(b) *Identification:* — The product affords protection to susceptible animals against rinderpest.

(c) *Moisture content:* — Not more than 1.0 per cent.

(d) *Safety Test:* — Each batch of vaccine shall be tested for safety in laboratory animals and cattle or buffalo calves as follows: —

(i) *Small animals:* — At least two guineapigs each weighing 300 g. to 450 g. and two adult rabbits each weighing 1 kg. to 1.5 kg. should be injected each with 1 ml. of 1:100 suspension of the vaccine subcutaneously and kept under observation for seven days. None of the animals should die. Alternatively, a batch of six white mice each weighing not less than 18 g. may be used, each mouse receiving 0.5 ml. of a dilution 1:100 suspension subcutaneously. None of the animals should die.

(ii) *Large animals:* — Either cattle of good grade of susceptibility (hill cattle) or buffalo calves may be employed. For each batch of vaccine, three animals should be injected subcutaneously with 1 ml. of 1:8000 dilution of the vaccine. These animals should be kept under observation for twelve to fourteen days. None of the animals should show any untowards reactions.

(e) *Sterility Test:* — Complies with the tests for sterility described under the general monograph on «Viral Vaccines».

(f) *Potency Test:* —The animals receiving 1 ml. 1:8000 dilution of vaccine used under safety test mentioned above and kept under observation for fourteen days, should be challenged with 1 ml. of 1 per cent suspension of stock Rinderpest Virulent virus. None of the animals should die of rinderpest within a period of ten days. This test serves as a short potency test for each of the batches.

For conducting a detailed potency test the following procedure may be followed: —

Dilution 1:8,000 1:12,000 and 1:16,000 shall be tested and for each dilution three susceptible cattle or buffalo calves should be used. Each animal is inoculated subcutaneously with 1 ml. of a dilution of the vaccine, followed twelve to fourteen days later with a standard challenge dose of virulent rinderpest bull virus containing in 1 ml. of a 1:100 suspension of spleen tissue. Two unvaccinated bovines, each receiving the same quantity of the challenge dose act as controls. These are kept under observation for fourteen days. The end point of protection titre is assessed on the death or survival rate and the dose contained in one gramme of vaccine calculated on the basis of 20 to 40 minimum protective doses being equivalent to one vaccinating dose.

(g) *Virulence and viability Test:* — Two to four goats each weighing not less than 18 kg. are injected with 2ml. of 1:100 suspension of the vaccine and kept under observation for ten days. These animals should show reaction characterised by pyrexia (rise of about 2°C anorexia and dullness.

5. **Labelling:** — Should comply with the requirements of «Labelling» as laid down in the general monograph on «Viral Vaccines».

6. **Storage:** — The vaccine may be expected to retain its potency for twelve months if stored at -15°C to -20°C or about three months if stored at 2°C to 4°C.

RINDERPREST LAPINISED VACCINE (LIVING)

1. **Synonym:** — Rabbit Adapted Cattle Plague Vaccine (Living) Lapinised Vaccine (Living).

2. **Definition:** — Rinderpest Lapinised Vaccine is a suspension of a modified living virus (e. g. Nakamura III Strain) prepared with the blood, spleen and mesenteries lymph glands of infected rabbits and is freeze dried.

3. **Preparation:** — Adult rabbits possibly from a known stock, each weighing not less than I kg. free from cocidiosis and snuffles, are injected intravenously with 1 ml. of a suitable dilution of a suspension of the stock, seed virus. Donor rabbits are sacrificed after a suitable period when the titre of the virus in the animals is expected to be maximum usually the third day.

Ten millilitres of blood is collected from each rabbit in a defibrinating flask under aseptic condition. Later the animals are sacrificed and the spleen and measentric lymph glands collected. Each rabbit is subjected to a thorough postmortem examination to observe lesions of rinderpest infection.

After harvesting, the blood and the organs (spleen and glands) are homogenised in a suitable proportion if necessary. Adequate quantities of penicillin and streptomicin may be added. The homogenised material is ampouled in suitable quantities and freeze dried.

4. **Standard:**

(a) *Description:* — Dark chocolate coloured mass.

(b) *Identification:* — This product affords protection to susceptible animals against rinderpest.

(c) *Moisture content:* — Not more than 1.0 per cent.

(d) *Safety Test:* — For testing a batch 2 guinea pigs each weighing not less than 300 g. are injected subsutaneously with 1 ml. of a 1:100 suspension, of the vaccine. Alternatively, a group of six white mice each weighing not less than 18 g. is used Each animal received subcutaneously 0.5 ml. of 1:100 suspension of the vaccine. None of the test animals should die within a period of seven days.

(e) Sterility test: — Should comply with the tests for sterility described in the general monograph on «Viral Vaccines». If antibiotics have been added the inoculum should be neutralised before doing the test.

(f) *Potency test:* — Dilutions 1:100, 1:200, 1:400 and 1:800 shall be tested and for each dilution 2 susceptible cattle (hill bulls) for buffalo calves should be used. Each animal is inoculated subcutaneously with 1 ml. of a dilution of the vaccine, followed twenty-one days later with a standard challenge dose of a virulent rinderpest bull virus contained in 1 ml. of a 1:100 suspension of spleen tissue. Two unvaccinated bovines; each receiving the same quantity of the challenge virus serve as controls. These animals are kept under observation for fourteen days. The end point of the protecting titre is assessed on the death or survival rate and the dose contained in one gramme of vaccine calculated on the basis of twenty minimum protective doses being equivalent to one vaccinating dose.

(g) *Virulence and Viability Tests:* — Four rabbits each weighing 1 to 1.5 kg. are injected subcutaneously with 1ml. of 1:100 suspension of the vaccine. The animals should react typically showing all the symptoms of rinderpest in rabbits.

5. **Labelling:** — Should comply with the requirements of «Labelling» as laid down in general monograph on «Viral Vaccines».

6. **Storage:** — The vaccine may be expected to retain its potency for six months if stored at -15°C to -20°C or about a month if stored at +2°C to 4°C.

RINDERPREST LAPINISED AVIANISED VACCINE (LIVING)

1. **Synonym:** — Lapinised Avianised Vaccine (Living).

2. **Definition:** — Rinderpest Lapinised Avianised Vaccine is a suspension of a modified live rinderpest virus of low virulence prepared either with the whole chick embryo or the viscera of the infected chick embryo.

3. **Preparation:** — Twelve or thirteen day old active chick embryos from a flock free from Salmonell pullorum infection are injected intravenously with a suitable dilution of the suspension of the stock seed virus in six per cent glucose solution. The embryos are incubated at 38.50°C for five days. At the end of this incubation period, eggs which show living embryos are selected for the preparation of the vaccine. The viscera of the chicks are harvested, care being taken to reject the gizzard and gall bladders. The material is homogenised in a blender with adequate quantities of antibiotics (penicillin an streptomycin added if necessary), and primary freeze dried done. This freeze dried material is ground into a fine powder, ampouled in suitable quantities and finally subjected to secondary freeze drying and sealed under vacum.

4. **Standard:**

(a) *Description:* — Pale cream or yellow coloured sterile powder.

(b) *Identification:* — This product affords good grade of immunity to susceptible animals against rinderpest.

(c) *Moisture content:* — Not more than 1.0 per cent.

(d) *Safety Test:* — For testing each batch, a group of six mice each weighing not less than 18 g. is used. Each mouse is injected subcutaneously with 0.5 ml. of a 1:100 suspension. Alternatively, two guinea pigs each weighing not less than 300 g. and two rabbits each weighing not less than 1 kg. are injected with 1 ml. of 1:100 suspension subcutaneously. These animals should not show any untoward reaction during the period of observation for seven days.

(e) *Sterility test:* — Should comply with the test for sterility as laid down in the general monograph on «Viral Vaccines».

(f) *Potency Test:* — Healthy highly susceptible cattle (hill bulls) for buffalo calves should be used for testing the potency of each batch of vaccine in suitable dilution. For each dilution two highly susceptible animals should be used. Each animal is inoculated subcutaneously with 1 ml. of a dilution of the vaccine, followed twenty-one to twenty-eight days later, with a standard challenge dose of a virulent rinderpest bull virus contained in 1 ml. of a 1:100 suspension of spleen tissue. Two unvaccinated bovines, each receiving the same quantity of the challenge virus serve as controls. All these animals are kept under observation for fourteen days. The end point of protective titre is assessed on the death or survival rate and the dose contained in one gramme of vaccine calculated on the basis of forty minimum protective doses being equivalent to one vaccinating dose.

5. **Labelling:** — Should comply with the requirements of «Labelling» as laid down in the general monograph on «Viral Vaccines».

6. **Storage and expiry date:**—The vaccine shall be expected to retain its potency for the period and at temperatures as specified below:

-15°C to -20°C — Six months.
2°C to 4°C — One month.

SHEEP AND GOAT POX VACCINE (LIVING)

1. **Synonym:** — Sheep Pox Vaccine. Goat Pox Vaccine.

2. **Definition:** — Sheep and Goat Pox Vaccine consists of the virus contained in the scabs collected from sheep artificially infected with the virus.

3. **Preparation:** — Healthy yearling sheep are infected artificially on the shaved portion of the abdomen with a suitable dilution of the suspension of the stock seed virus 50 per cent glycerine saline solution. The material from the semi-dried areas where the pock lesions are evident is collected and dried over calcium chloride or phosphorous pentoxide under vaccum. Dry scabs are powdered, sieved, ampouled in suitable quantities and sealed.

4. **Standard:**

(a) *Description:* — Light cream coloured powder.

(b) *Identification:* — This product when applied to scarified area of the skin of the sheep or goats produces characteristic local lesions of pox and should afford protection to sheep and goat against sheep and Goat Pox.

(c) *Safety Test:* — Two rabbits each weighing not less than 1 kg. are injected subcutaneously each with 1 ml. of a 1.100 dilution of the vaccine in normal saline solution. These animals are observed for fourteen days. The animals should remain normal.

(d) *Sterility Test:* — Complies with the tests for sterility described under the general monograph on 'Viral Vaccines'.

(e) *Potency Test:* — Four yeadling sheep are inoculated with 1:100 suspension of the vaccine in 50 per cent glycerin saline on a scarified area on the abdomen. Fourteen days later, these and two more suceptible sheep are inoculated by by the same method with stock virus and observed for a period of fourteen days. The control animals should develop typical lesions of pox and the vaccinated animals should remain normal.

5. **Labelling:** — Should comply with the requirements of (Labelling) as laid down in the general monograph on 'Viral Vaccines'.

6. **Storage and expiry date:**—The vaccine shall be expected to retain its potency for period at temperatures as specified below: —

-15°C to -20°C — Twenty months.
-2°C to 4°C — Three months.
Room Temp. — Fifteen days.

FOWL SPIROCHAETOSIS VACCINE (CHICK EMBRYO ORGIN)

1. **Synonym:** — Tick Fever Vaccine.

2. **Definition:** — The vaccine consists of a merthiolated suspension of choricallantoric membrane, internal viscera and blood of chick embryos infected with a vaccine strain of spirochaetes and freeze dried.

3. **Preparation:**—Eleven days old developing chick embryos are infected with 0.2 ml. of sterile fresh blood containing spirochaets via the choricallantoic membrane. The inoculated embryos are incubated at 37°C and candled daily and the dead ones are discarded. On the seventh day the living embryos are chilled in the refrigerator for two hours. The chilled embryos are harvested separately and necrotic lesions in live noted. Representative samples of blood should be examined for teaming spirochaetes. The internal viscera, cholioallantoric membranes and the blood are collected. The material is pooled, weighed and held in deep freeze at 15 to 20°C for a period of one week. Thereafter the material is blended with equal quantity of Marthiolate (final concentration of merthiolate in the suspension should be 1:10,000) throughly for three times, each time the motor running at full speed and the vaccine is ampouled in 2 ml. quantities and freeze dried.

4. **Standard:**

(a) *Description:* — Light brownish scales.

(b) *Identification:* — The vaccine affords protection when inoculated into the fowls against spirocheetosis.

(c) *Moisture content:* — The moisture content should not exceed 1.0 per cent.

(d) *Safety and potency test:* — Six healthy cockerals ten to twelve weeks old are used for this purpose. Each ampoule of vaccine is reconstituted in 10 ml. of cold distilled water and the six cockerals are injected intramuscularly each with 1 ml. of the reconstituted vaccine and the birds are observed for a period of ten days and the vaccinated birds should remain normal throughout the period of observation. The vaccinated birds are challenged with 0.2 ml. intramuscularly with virulent spirochaete blood along with two susceptible controls. Temperature and blood smear examination of the challenged birds and controls should be carried out daily for a period of ten days. The blood smears of vaccinated birds should remain negative for spirochaetes during the entire period of observation. The controls should react and show spirochaetes in the blood and ie.

(e) *Sterility Test:* — complies with the tests for sterility described in the general monograph on 'Bacterial vaccine'.

5. **Labelling:** — Should comply with the requirements of 'Labelling as laid down in the general monograph on 'Bacterial Vaccine'.

6. **Storage:** — The vaccine when stored at — 15°C to — 20°C may be expected to retain the potency for about one year and about two monthe if stored in refrigerator at 2° to 4°C.

SWINE FEVER VACCINE CRYSTAL VIOLET

1. **Synonym:** — Crystal Violet Swine fever vaccine, Hog Cholera vaccine.

2. **Definition:** — Swine fever vaccine, crystal violet is a suspension of blood of swine that have been infected with a suitable virulent antigenic strain of swine fever virus, inactivated with 0.25 per cent crystal vilet ethylene glycol at 37°C for fourteen days.

3. **Preparation:** — Susceptivle healthy pigs of six to seven months of age belonging to a well established strain or breed are used. Body weight of these animals at this age may vary according to the breed but optimum weight is considered as between 75 to 100 kg. Animals used for production may be procured from well established farms and kept under quarantine for fourteen days. These are injected intramuscularly with a suitable dilution of the suspension of the virulent blood viruses. Bleeding of the clinically injected animals is carried out on the sixth day. The defibrinated blood from each animal is strained and stored separately in sterile glass containers. To the four parts of defibrinated blood, one part of 0.25 per cent crystal violet-ethylene glycol is added and the suspension after thorough mixing, is stored at 37°C (-0.5) for two weeks. The product is filled in 20 ml. volumes in sterile vials and labelled on the completion of tests.

4. **Standard:**

(a) *Description:* — Very dark violet suspension.

(b) *Identification:*—This product affords protection against swine fever but not against African Seine Fever.

(c) *Safety Test:* — Two young pigs weighing about 15 to 30 Kg. are injected subcutaneously each with 10 ml. of the vaccine batch to be tested. In addition, one unvaccinated susceptible pig is placed in contact.

(d) *Sterility Test:* — Should comply with the test for sterility described under the general monograph in «Viral Vaccines».

(e) *Abnormal toxicity test:* — Two guinea pigs are given 1 ml. of vaccine intramuscularly.

Two guinea pigs are given 2 ml. of the Vaccine intraperitoneally.

Two mice are given 0.5 ml. of the vaccine subcutaneously.

(f) *Potency Test:* — Four susceptible pigs weighing between 20-30 kg. are injected with 5 ml. of the vaccine subcutaneously. After twenty-one days these are challenged with 1 ml. of suitable dilution of the challenge virus subcutaneously. The dose must contain at least 1000 minimum infective doses. At least two control pigs should be used.

5. **Labelling:** — Should comply with the requirements of «Labelling» as laid down in the general monograph on «Viral Vaccines».

6. **Storage:** — The vaccine may be expected to retain its potency for twelve months if stored in refrigerator at 2°C to 4°C.

SWINE FEVER VACCINE LAPINISED (LIVING)

1. **Synonym:** — Lapinised swine fever vaccine, freeze dried lapinised swine fever vaccine.

2. **Definition:** — Swine fever lapinised consists of the suspension of a modified live swine fever virus prepared from spleens of infected rabbits and is freeze dried.

3. **Preparation:** — Healthy adult rabbits weighing approximately 1000 gms. or over, free coccidiosis snuffles etc. are injected intravenously with a suitable dose of a dilution of the modified rabbit adapted virus. Rabbits are sacrificed at the hight of reaction and spleens are collected with sterile precautions. The collection is later homogenised in a blender using ten per cent yolk phosphate buffer as a diluent. The suspension is ampouled in 0.5 ml. quantities and freeze dried.

4. **Standard:**

(a) *Description:* — Light scales.

(b) *Identification:* — This product affords protection against swine fever.

(c) *Moisture content:* — The moisture content should not exceed 1.0 per cent.

(d) *Safety test:* — Six mice are injected each with 0.5 ml. of a 1:100 suspension of the vaccine. These are kept under observation for seven days. Non should die.

(e) *Viability test:* — Two healthy rabbits are injected intramuscularly with 1 ml. of 1:100 suspension of the vaccine. These animals thermal reaction.

(f) *Sterility test:* — Should comply with the test for sterility described under general monograph on «Viral Vaccines».

(g) *Potency Test:* — The vaccine batch under test should be tested on susceptible healthy pigs weighing between 20-30 kg. Two animals for each dilution may be used. The dilutions tested are 1:10, 1:25, 1:50 and 1:100. One millilitre of each of these dilutions is injected in subcutaneously. One healthy, susceptible, unvaccinated in contact animal should be kept along with the vaccinated animals.

Fourteen to twenty-one days later these animals along with two controls are injected subcutaneously with 1 ml. of the challenge virus containing at least 1000 minimum infective doses.

5. **Labelling:** — Should comply with the requirements of «Labelling» as laid down in the general monograph on «Viral Vaccines».

6. **Storage:** — The vaccine may be expected to retain its potency for six months if stored at temperature ranging between 10° C to 15° C and for seven months at 2° C to 4° C in the refrigerator.

PART II — ANTISERA

PROVISIONS APPLICABLE TO THE PRODUCTION OF ALL SERA FROM LIVING ANIMALS

1. **Definition:** — (i) This part of the Schedule applies to antibacterial sera, anti-viral sera and anti-toxic sera which are prepared by injecting bacteria or viruses or their products into buffallo-bulls or other suitable animals so as to produce active immunity which is manifested by the formation of anti-body.

(ii) For the purpose of this part of the Schedule and antiserum means sterile liquid antiserum concentrated and unconcentrated, solutions of globulins or their derivatives or solid forms which can be reconstituted when necessary.

2. **Staff of Establishment:** — The establishment shall be under the direction and control of a competent expert in bacteriology and serology with adequate training in immunology and standardisation of biological products and know-

ledge of animal management. He shall be assisted by a staff adequate for carrying out the tests required during the course of preparation of the sera and standardisation of the finished products.

3. **Proper Name:** — The proper name of the antiserum shall be the recognised scientific name of the diseases or its causative organism or some generally recognised abbreviations thereof preceded by the prefix «anti», and followed by the word «serum» as for example, «Anti-anthrax serum». The proper name of any antitoxin may be formed from the word «Anti-toxin» preceded by the name of the organism from which the toxin was prepared, and followed, if desired, by a term indicating the source or the strain of that organism provided where there is no special provision in the Schedule, the name as approved by the licensing authority may be adopted.

4. **Records:** —

(1) The permanent records which the licensee is required to keep shall include the following particulars:

(a) As to the culture — Evidence of the identity and specificity of the cultures.

(b) As to the procedure used in immunising the animals:

(i) The method of preparing the cultures antigent used for immunisation.

(ii) The dosage and methods employed in administering the culture or antigen.

(iii) The period in the course of immunisation at which blood is withdrawn for the preparation of the serum.

(c) Any test which may have been applied to the serum to determine its content of specific antibodies or its specific antibodies or its specific therapeutics potency and purity.

(2) If the licensee desired to treat the performance of any tests recorded under sub-paragraph (i) (c) of this paragraph as determining the date of completion of manufacture for the purpose of rule 109 he shall submit full particulars of the purpose test to the licensing authority and obtain his approval.

5. **Cultures:** — The cultures used in immunising the animals shall be at all times open to inspection, and specimens shall be furnished for examination at the request of the licensing authority.

6. **Quantity:** —

(a) Any antiserum shall be issued for veterinary use in the form of either,

(i) Actual serum, i. e., the liquid product of decantation of the coagulated blood or plasma without any addition, other than antiseptic or substraction, or

(ii) A solution of the purified serum proteins containing the specific anitibodies.

(b) At the time of issue, the liquid shall be clear or show at the most a slight opalescene or precipitate. Preparations of the natural serum shall not contain more than 10 per cent of solid matter. A solution of serum protein shall not contain more than 20 per cent of solid matter.

7. **Precautions to be observed in preparation:** — (i) Laboratories where sera are exposed to the air in the course of the process of preparation must be separated by a sufficient distance from stables and animal houses to avoid the risk of serial contamination with bacteria from animal excreta, and must be rendered fly-proof to prevent such contamination by insects. Such laboratories must have impervious walls and floors and must be capable of being readily disinfected when necessary.

(ii) A special room with impervious walls must be provided for the collection of blood from the living animals.

(iii) An efficient system of manure removal must be used which will prevent its accumulation in the vicinity of any room were blood or serum is collected or handled.

(iv) An adequate number of sterilizers must be provided for the sterilization of all glassware or other apparatus with which the serum may come into contact in the course of its preparation.

(v) All processes to which the serum is subjected during and after the collection from the animals, must be designed to preserve its sterility, but in the case of artificially concentrated sera, it shall suffice that the process of concentration is conducted with scrupulous cleanliness and in such a manner also avoid unecessary dangerous contamination.

(vi) The laboratories in which the testing of the sera for potency, sterility and freedom from abnormal toxicity are carried out must be adequate for the purpose. An adequate supply of animals for use in such tests and suitable housing for such animals must be provided.

(vii) Provision must made for complying with any special conditions which may be laid down in the Schedule relating to the production an issue of the particular serum, in respect of which the licence is granted.

8. **Unhealthy or Infected Animals:** — If an animal used in the production of sera is found to be suffering from an infection except one produced by living organisms against which it is being immunized, or shows signs of serious or persistent ill health not reasonably attributable to the process of immunisation, the licensee shall immediately report the matter to the licensing authority and shall, if the authority orders an inspection and the inspection so directs, cause such animals to be killed and a postmortem examination of it to be made, and take steps to prevent any serum obtained from the animal being sold or offered for sale until permission is given by the licensing authority. If the result of the postmortem is such as to bring under suspicion, the health of any of the other animals used for the production of sera, the licensing authority may prohibit the use of those animals for the production of sera or may take such other steps as may be necessary to prevent the issue of sera which may be dangerous to animal health.

Provided in the case of emergency, the person in charge of the establishment may order the destruction of animal used in the production of sera and suspecious of infection, and shall in that case given notice fortwith to the licensing authority and shall permit an inspector to be present at the posmortem examination.

9. **Conditions and Housing of animals:** — (i) The animals used in the production of sera should be adequately housed under hygienic environments.

(ii) Only healthy animals free from disease should be used in the preparation of sera.

(iii) Every animal intended to be used as the source of serum must be subjected to a period of observation in quarantine for at least seven days before being admitted to the animal sheds in which the serum yielding animals are housed.

(iv) In case of horses and other equidae, every animal used as source of serum shall either be actively immunized against tetanus or shall be passively immunized against the disease by injection of tetanus antitixin in such doses as to ensure the constant presence of that antitoxin in the blood during the whole period of the use of the animal as a source of serum.

ANTISERA AND THEIR GENERAL STANDARD

Antisera contain the immune substance that have a specific prophylactic or thereapeutic action when injected into animals exposed to or suffering from a disease due to a specific micro-organism or its toxin. Antisera are classified into three groups.

(i) Antitoxic sera (Antitoxine).
(ii) Antibacterial sera.
(iii) Antiviral sera.

Antisera are usually issued in an unconcentrated form for animal use but may be concentrated and also freeze dried. However, for the purpose of the Schedule the word «antisera» is also used for the unconcentrated liquid sera only. A suitable bacteriostatic agent in a concentration sufficient to prevent the growth of micro-organisms is added to the liquid serum.

GENERAL STANDARD

(1) **Description:** — Liquid native or unconcentrated antisera are yellow or yellowish brown in colour. They are initially transparent but may become turbid with age. They are almost odourless, except for the odour of any bacteriostatic agent that may have been added.

(2) **Identification:** — The test for identity is described in the individual monograph.

(3) **Acidity or Alkalinty:** — All native antisera have a PH of 7.0 to 8.5.

(4) **Abnormal Toxicity:** — All antisera shall comply with the following tests for freedom from abnormal toxicity:

(a) Two healthy mice each weighing not less than 18 g. are injected subcutaneously each with 0.5 ml. of the sample and observed for five days. None of the mice should show any abnormal reaction or die.

(b) Two healthy guinea-pigs each weighing 300 g. to 450 g. are injected subcutaneously each with 5 ml. of the sample and observed for seven days. None of the guinea-pigs should show any abnormal reaction or die.

(5) **Sterility:** — All antisera shall comply with the tests for sterility described in rules 115 to 119.

(6) **Potency:** — The potency of each preparation, when the available methods permit, is determined by the appropriate biological assay, and it is described under the individual monograph.

(7) **Total Solids:** — Native antisera should not contain more than 10 per cent solid matter.

(8) **Labelling:** — Should comply with the provisions for 'Labelling' as laid down for 'Bacterial Vaccines'.

(9) **Storage:** — Liquid preparations of antisera shall be stored, protected from light at temperature between 2°C to 4°C and shall not be frozen.

(10) **Date of Manufacture:** — The date of manufacture shall be unless otherwise specified in the individual monograph in this part as defined in clause (b) of sub-rule (3) of rule 109.

(11) **Containers:** — All antisera are distributed in sterilised containers of a material which is inert towards the substance and which are sealed to exclude micro-organisms.

(12) **Expiry Date:** — The expiry date of potency of all sera shall not more than twenty-four months after the date of manufacture.

ANTI-ANTHRAX SERUM

1. **Synonym:** — Bacillus Anthracis Antiserum.

2. **Definition:** — Anti-anthrax serum is the serum of animals that confers a specific protection against *bacillus anthracis.*

3. **Preparation:** — The antiserum may be prepared in buffalo bulls after repeated injections of cultures of *B. anthracis* of a virulent strain.

4. **Standard:** — It complies with the requirements in the general provisions for antisera under Description, Acidity or Alkalinity, Abnormal Toxicity, Sterility, Solids, Labelling, Storage and Expiry date.

(i) *Identification*: *It* protects animals against infection with *B. Anthracis.*

ANTI-BLACK QUARTER SERUM

1. **Synonym:** — Blackleg Antiserum, Clostridium Chauvoei--Antiserum.

2. **Definition:** — Anti-Blacquarter serum is the serum of suitable animals containing the substances that have a specific neutralising effect on *Colostridium Chauvoei.*

3. **Preparation:** — It is prepared by injecting subcutaneously or or intramuscularly increasing doses of formalised cultures of *Cl. Chauvoie,* into buffalo bulls.

4. **Standards:** — It complies winth the requirements described in the general provisions for antisera under Description, Acidity or Alkalinity, Abnormal toxicity, Sterility, Solids, Labelling, Storage and Expiry Date.

5. **Identification:** — It protects susceptible animals against infection with virulent strains of *Cl. Chauvoei.*

ANTI-FOWL-CHOLERA SERUM

1. **Synonym:** — Pasteuralla Septica Antiserum (Avian).

2. **Definition:** — Fowl Cholera Antiserum is the serum of animals containing the substances that confer a specific protection to fowls against virulent strain of Pasteurella Septics (Avian).

3. **Preparation:** — Antiserum is prepared from buffalo, bulls after they have been subjected to an injection of killed cultures of virulent strain of pasteurella Septica (Avian) followed by injections of living cultures of the same organism.

4. **Standard:** — It complies with the requirements described in the general provision for antisera under Description, Acidity or Alkalinity, Abnormal toxicity, Sterility, Solids, Labelling, Storage and Expiry Date.

Identification: — It protects susceptible fowls against infection with Pasteurella Septica (Avian) and its homologous strains.

ANTI-HAEMORRHAGIC SEPTICAEMIA SERUM

1. **Synonym:** — Pasteurella Septica Antiserum.

2. **Definition:** — Anti-Haemorrhagic Septicaemia Serum is the serum of animals containing the substances that confer a specific protection to susceptible antimals against virulent strains of *Paseteurella Septica.*

3. **Preparation:** — The antiserum is prepared from buffalo -bulls after they have been subjected to repeated injections of formolished cultures of standard strain *Pasteurella Septica* with adjuvants, followed by suitable doses of virulent culture of the organism.

4. **Standard:** — It complies with the requirements described in the general provisions for antiserum under Description, Acidity or Alkalinity, Abnormal toxicity, Sterility, Solids, Labelling, Storage and Expiry Date.

Identification: — It protects susceptible animals against infection with homologous strains of *Pasteurella Septica.*

ANTI-RINDERPEST SERUM

1. **Synonym:** — Cattle Plague Antiserum.

2. **Definition:** — Anti-rinderpest serum in the serum of buffalo bulls containing the substances that confer a specific immunity to susceptible animals against virulent strains of the virus of rinderpest.

3. **Preparation:** — The antiserum is prepared from buffaloes who have reacted to a dose of virulent rinderpest virus, which is injected simultaneously with a predetermined quantity of antirinderpest serum so as to control the severity of the reaction (serum-simultaneous-method).

4. **Standard:** — It complies with the requirements described in the general provisions for antisera under Description, Acidity or Alkalinity, Abnormal toxicity, Slids, Labelling Storage and Expiry date.

(i) *Identification:* — It protects susceptible animals against rinderpest.

(ii) *Potency:* — Five Buffalo-calves of about one year of age in good condition are used for the test. Three are injected subscutaneously with the anti-rinderpest, serum under test at the rate of 10 ml. per 46 kg. body weight, subject to a minimum of 20 ml. per animal. These together with the two remaining, are simultaneously injected subcutaneously at a different site with 1 ml. of a 1:100 dilution of spleen suspension of virulent bull-virus.

The animals should be observed for fourteen days during which time the serum treated animals should exhibit no symptoms of rinderpest other than rise in temperature and slight intestinal disturbances, while the controls develop more severe symptoms or die.

SALMONELIA PULLERUM ANTI-SERUM

1. **Synonym:** — Salmonella Pullorum anti-serum.

2. **Definition:** — *Salmonella Pullorum* anti serum is the sera from fowls and contains antibodies against *Salmonella Pullorum.* It is used for standarizing batches of *Salmonella Pullorum* antigens and also used as a control along with the sera suspected for pullorum disease.

3. **Preparation:** — The serum is prepared after intravenous inoculation with smooth culture suspension of *Salmonealla Pullorum* in healthy birds.

4. **Standards:** — It complies with the requirements in the general provision for antisera under description, Acidity, Alkalinity, Sterility, solids, labelling, storage and expiry date.

5. **Identification:** — It should give positive agglutination with Salmonella pullorum antigen.

STANDARD ANTI-BRUCELLA ABORTUS SERUM

1. **Synonym:** — National counterpart of standard anti-Brucella abortus serum.

2. **Definition:** — Standard anti-Brucella abortus serum is the serum which contains 1000 International Units (I. U.) per ml. and is used for standardizing batches of brucella antigens and is also used as a control along with the sera suspected for brucellosis.

3. **Preparation:** — The serum is prepared after intravenous inoculation of suspension of smooth culture of B. abortus (strain 99) in rabbits or cattle and subsequently diluting it suitably with brucella-free healthy serum such that when tested with standardized Brucella abortus tube test antigen, it gives 50% agglutination at 1/500 final serum dilution.

4. **Standard:** — It complies with the requirements in the general provision for anti-sera under description, acidity, alkalinity, sterility, solids, labelling storage and expiry date.

Identification: — It should give aggultination with brucella antigen.

PART III — DIAGNOSTIC ANTIGENS

PROVISIONS APPLICABLE TO THE MANUFACTURE AND STANDARDISATION OF DIAGNOSTIC AGENTS (DACTERIAL ORIGIN)

1. **Definition:** — This part of the Schedule applies to reagents of bacterial origin employed for various tests.

2. **Staff of Establishment:** — A competent expert in bacteriology with sufficient experience in the manufacture and standardisation of veterinary biological products shall be in charge of the establishment responsible for the production of various diagnostic agents of bacterial origin and he may be assisted by a staff adequate for carrying out the tests required during the preparation and standardisation of various diagnostic agents.

3. **Proper Name:** — The proper name of any diagnostic agent is the name of micro-organism from which it is made, followed by the word 'antigen' unless the Schedule otherwise provides, or, it may be derived from the name of the organism responsible for causation of the disease or if there is no special provision in the Schedule, the name approved by the Licensing authority. In the case of the undermentioned preparations the proper name of the diagnostic agent may be as follows:

1. Abortus Bang Ring (A. B. R.) ANTIGEN.
2. Brucella Abortus Coloured Antigen.
3. Brucella Abortus Plain Antigen.
4. Johnin.
5. Mallein.
6. Salmonella Abortus Equi 'H' Antigen.
7. Salmonella Pullorum Coloured Antigen.
8. Salmonella Pullorum Plain Antigen.
9. Tuberculin.

4. **Records:** — Cultures used in the preparation of diagnostic agents of bacterial origin must, before being manipulated into an agent be thoroughly tested for identity by the generally accepted tests applicabl to the particular micro-organism. The permanent record which the licensee is required to keep shall amongst other include a record of the origin, properties and characteristics of the cultures.

5. **Preparation:** — Diagnostic agents of bacterial origin are prepared from selected cultures after their careful examination for the identity, specificity, purity and antigenicity. They may be prepared in the following manner.

(a) *Formolised antigens:* The selected pure culture stain grown in a suitable medium at an optimum temperature for an appropriate period. The pure growth is then exposed to the action of a solution of Formaldehyde I. P. in a suitable concentration and at an appropriate temperature for a suitable period.

(b) In some cases, the diagnostic agents are prepared by growing the organisms on suitable media and then deriving specific protein constitutents of the bacteria by various methods.

6. **General Standard:** —

(a) *Description:* Diagnostic agents may be clear opalescent or coloured liquids.

(b) *Identification:* Same exhibit specific aggulatnation when mixed with the serum of the animals infected with homologous organisms and others when injected into the animal body in appropriate doses cause specific reactions like hypersensitiveness, local and general reaction, if the animal is infected with the homologous organisms.

(c) *Sterility Test:* All antigens shall be tested for sterility in accordance with rules 114 to 119.

(d) *Standardisation:* It is carried out either by determining the definite cell concentration in the product or by observing the general and local reactions in healthy and artificially infected animals with various standard dilutions of the product.

7. **Labelling:** — As under general provision for the bacterial vaccines with the addition that it is meant for diagnostic purposes only.

8. **Storage:** — All antigens are stored, protected from light at a temperature between 2° C to 4° C.

9. **Date of Manufacture:** — The date of manufacture shall be unless otherwise specified in the individual monograph in this part as defined in clause (b) of sub-rule (3) of rule 109.

ABORTUS BANG RING (ABR) ANTIGEN

*1. **Synonym:** — Milk Ring Test Antigen.

2. **Definition:** — The antigen is a suspension of pure growth culture of standard strain of *Brucella abortus* strain 99 strained supravitally with 2, 3, 5, triphenyl tetrazolium chloride suspended in 0.85 per cent saline containing 1 per cent glycerol and 1 per cent phenol.

3. **Preparation:** — Smooth strain of **Brucella abortus** strain 99 is grown on potato influsion agar for 48 to 72 hours in Roux flasks, at 37° C. Condensation fluid if any is pipetted off before washing. Each flask is washed with about 20 ml. of normal saline. The pooled washing is filtered through a gauze and the filterate is collected in a measuring cylinder. To every 500 ml. of the filtrate 1 g. of 2, 3, 5, triphenyl tetrazolium chloride is added immediately. The container is shaken for thirty minutes till the tetrazolium salt is dissolved. The product is taken out and kept at 37° C for two hours. After incubation the product is heated at 65° C in a water bath for thirty minutes. It is cooled and centrifuged at 3000 r.p.m. for one hour. The supernatant is pipetted off and sediment is suspended in normal saline containing 1 per cent glycerol and 1 per cent phenol and filtered through sterile cotton wool. This forms concentrated antigen.

STANDARDIZATION OF THE STRAINED ANTIGEN

An aliquot portion of the microbial suspension stained with phenyltetrazoldium is taken, representing the initial undiluted suspension. 1 ml. per tube of this initial undiluted stained suspension is added to six test-tubes, followed by increasing quantities of the glycerolphenol diluent as follows: —

	UNDILUTED STAINED SUSPENSION	Diluent
Tube 1	1	0.6
2	1	0.8
3	1	1.0
4	1	1.2
5	1	1.4
6	1	1.6

The contents of each tube are then diluted tenfold with the same diluent and srrve as antigen for a tube agglutination test with the Standard Serum (or its national counterpart). In this way, six sero-reactions will be carried out, During this procedure, the concentrated strained microbial suspension should be kept in the refrigerator at 4°C.

The agglutination reaction are read after forty-eight hours' reacts at the agglutination litre of the Standard Serum, previously, determined with the usual unstained antigen in the

tube test, corresponds to the correct dilution of the standard artigen.

The next step, therefore, is to dilute the concertrated stained suspension to the same extent as the tube whose tenfold dilution has given the correct agglutination titre, i. e., the concertration of antigen in the tube before the tenfold dilution had been made.

4. Standard: —

(a) *Description:* — It is a red colour liquid containing dead bacteria in suspension.

(b) *Identification::* — It shows formation of a specific oherry-red coloured ring in the cream layer when mixed with popled samples of milk taken from animals suffering from brucellosis.

(c) *Sterility test:* — Should comply with the tests for sterility described in the general monograph on 'Diagnostic Antigen'. The tests shall, however, be done before colouring.

5. Lobelling and Storage: — Should comply with the requirements of «Labelling» and «Storage» as laid down in the general monograph on «Diagnostic Antigen».

6. Expiry Date: — The date of expiry of potency shall be not more than nine months from the date of manufacture when stored at 2°C to 4°C.

BRUCELLA ABORTUS COLOURED ANTIGEN

1. Synonym: — Brucella abortus Cotton Strain 99 coloured Antigen.

2. Definition: — Brucella Abortus Coloured Antigen is a suspension of pure smooth cultures of Brucella abortus strain 99 in phenolised glycerine saline, the bacteria being coloured by the addition of crystal violet and brilliant green. This antigen is used for plate test for serological diagnosis of brucella infection.

3. Preparation: — Seventy-two hours old groth of Brucella Abortus stain ninety-nine in smooth from on potato infusion agar medium in Roux flasks is washed with phenolised glycertinesaline (containing 12 per cent sodium chloride, 20 per cent glycerine and 0.5 per cent phonol). The washed growth is filtered through a pad of absorbent cotton wool and the suspension is coloured by the addition of 1 ml. each of 1 per cent aqueous solution of crystal violet and brilliant green for every 250 ml., of the suspension. The product is heated for sixty minutes in a water bath at 60°C before it is standardised.

4. Standards: —

(a) *Description:* — It is a greenish violet liquid containing dead bacteria in suspension.

(b) *Identification:* — It gives specific agglutination when mixed with the serum of the animal with brucella organism.

(c) *Sterility Test:* — Should comply with the tests for sterility described in the general monograph on 'Diagnostic Antigens'.

(d) *Standardisation:* — 0.5 ml. of the antigen is mixed with 4.5 ml. of normal saline solution in Hopkins graduated tube. The mixture is centrifuged at 3000 r. p. m. for sixty minutes and the percentage of bacterial cells present in the original antigen is assessed by noting the height of the cell deposit. The antigen is then standardised so as to contain 10 per cent cell deposit.

5. Labelling and Storage: — Should comply with the requirements of «Labelling» and «Storage» as laid down in the general monograph on «Diagnostic Antigens».

6. Expiry Date: — The date of expiry of potency shall be not more than nine months from the date of manufacture when stored at 2°C to 4°C.

BRUCELLA ABORTUS PLAIN ANTIGEN

1. Synonym: — Brucella Abortus Strain 99 Plain Antigen.

2. Definition: — Brucella Abortus Plain Antigen is a suspension of a pure smooth culture of Brucella abortus strain 99 in phenol-saline.

3. Preparation: — Seventy-two hours old growth of Br. Abortus strain 99 in smooth form on potato infusion agar medium in Roux flasks is washed with normal saline solution. The washed growth is filtered through a pad of absorbant cotton wool and the suspension instead at 60°C for sixty minutes in a water bath to kill the organisms. It is then preserved by the addition of phenol in a final concentration 0.5 per cent.

4. Standard: —

(a) *Description:* — An opalescent liquid containing dead bacteria in suspension.

(b) *Identification:* — It gives specific agglutination when mixed with the serum of animals infected with brucella organism.

(c) *Sterility Test:* — Should comply with the tests for sterility described in the general monograph on «Diagnostic Antigen».

(d) *Standardisation:* — Mix the concentrated antigen well and dilute 1 ml. with 0.5% phenol saline until it corresponds to about tube four of Brown's opacity tubes. Further dilutions of the antigen adjusted to opacity tube No. 4 are made. The particular dilution that gives 50 per cent agglutination with anti-brucella abortus serum (containing 1000 International Units) at 1:500 final serum dilution, is assessed as the diluting factor for the concentrated antigen. The bulk of the contrated antigen is accordingly diluted for issue as standards antigen.

5. Labelling and Storage: — Should comply with the requirements of «Labelling and Storage» as laid down in the general monograph on «Diagnostic Antigen.

6. Expiry Date: — The date of expiry of potency shall be not more than nine months from the date of manufacture when stored at 2°C to 4°C.

JOHNIN

1. Definition: — Johnin is a preparation of a fluid medium in which *Mycobacterium paratuberculosis* has been grown in artificial culture and which has been freed by filtration from the bacilli.

2. Preparation: — Young culture of culture of selected strain of *Myco paratuberculosis* of bovine origin is grown on synthetic medium and incubated at 37°C for ten to twelve weeks. Flasks showing lucurient and pure Growth are steemed for three hours thereafter kept at room temperature overnight. The contents are filtered through fine meshed sieve. The filtrate is concentrated over a steam bath to one-tenth of its original volume and kept in cold storage for fourteen days before being filtered through Seitz filter. The product is dispensed in ampoules and herme tically sealed.

3. Standards: —

(a) *Description:* — A yellowish brown to brownish liquid.

(b) *Identification:* — It produces hot, painful and oedemateus swelling at the site of inculation in animals infected with Myco-paratuberculosis organism.

(c) *Sterility Test:* — Should comply with the test for sterility described in the general monograph on «Diagnostic Antigens».

(d) *Potency Test:* Two animals, previously infected with Myco-paratuberculosis and two healthy animals are each injected intradermally in the neck region with 0.1 ml. of the product. Forty-eight hours later, the injection is repeated at the same site. The product should produce a typical reaction in the infected animals in the form of a hot, painful and cedemetous swelling at the site of inoculation persistent for at least forty-eight hours after the second injection. Centrol animals should not show such reaction.

4. Labelling and Storage: — Should comply with the requirements of «Labelling» and «Storage» as laid down in the general monograph on «Diagnostic Antigens».

5. Expiry Date: — The date of expiry of potency shall be not more than two years from the date of manufacture when stored at 2°C to 4°C.

MALLEINS

1. Definition: — (i) Malleins are preparations of fluid media in which the *Actinobacillus mellei* has been grown in a artificial culture and which have been freed by filtration from the bacilli.

(ii) For the purposes of this Schedule malleins are classified into (*a*) Mallein-subcutaneous and (*b*) Mallein intredermo-palpebral (I. D. P.).

2. **Preparation:** —

(*a*) *Mallein Subcutaneous:* — Three to four weeks old pure growth of standard strain of A. mallei grown on synthetic medium is steamed for one hour in Koch's steam sterilizer One part of 5 per cent phenol solution is added to every nine part of the dead culture which is then filtered through Seitz filter.

(*b*) *Mallein Concentrated:* — The procedure is the same as for Mellein Subcutaneous expect, that the filtrate is evaporated in porcelain dish over steam to half the original volume before addition of phenol. Five per cent phenol solution is added in sufficient quantity to the concentrated product, to give a final concentration of 0.5 per cent.

3. **Standards:** —

(*a*) *Description:* A yellowish to brown viscous liquid.

(*b*) *Identification:* — It produces hot tense, painful swelling when injected into the animals infected with *A. malle.* orga nisms.

(*c*) *Sterility test:* — Should comply with the tests for sterility described in the general monograph on «Diagnostic Antigens».

(*d*) *Potency test:* —

(*i*) *Mallein subcutaneous:* — Two ponies, previously sensitised with A. Mallei and controls, are injected each with 1 ml. of the product subcutaneously in the neck region. The animals are observed for local reaction and rise in temperature. Local reaction is manifested by a hot, tense, painful swelling becoming prominent within twenty-four hours. The rise in temperature is observed by recording the body temperature at the time of inoculation and subsequently at short intervals. A rise in temperature of 1°C or more above normal is indicative of infection.

(ii) *Mallein Intra-dermo-Palpebral (I. D. F.):* — Two ponies previously sensitized with A. mallei and two healthy ponies are injected intradermally with 0.2 ml. of the product near the rim of the lower eye lid of one eye. Typical reactions such as painful swelling of the palpebral tissue with mucopurulent discharge from the eye is indicative of infection. The two healthy ponies should not show such reactions.

Similar test in other eye is performed with a previously determined patient mallein using as a standard. When the local reactions produced by intradermo palpebra infections of the two preparations are comparable the bach is passed for issue.

4. **Labelling and Storage:** — Should comply with the requirement of «Labelling» and «Storage» as laid down in the general monograph on «Diagnostic Antigen».

5. **Expiry Date:** — The date of expiry of potency shall be not more than two years from the date of manufacture when stored at 2°C to 4°C.

SALMONELLA ABORTUS EQUI 'H' ANTIGEN

1. **Synonym:** — Equine Abortion Diagnostic Antigen.

2. **Definition:** — Salmonella Abortus Equi Antigen is suspension of a pure smooth culture of actively *motile salmonella Abortus equi* in formal saline.

3. **Preparation:** — Standard stain of S. abortus equi is grown on nutrient agar in Roux flasks at 37° for twenty-four hours. The pure growth in Roux flaks is washed with normal saline and diluted to contain approximately 800 million organisms per ml. Solution of Farmaldehde I. P. is added to give a final concentration 0.5 per cent and the formolised product is incubated at 37°C for twenty-four hours. The final product is dispensed in suitable containers.

4. **Standards:** —

(*a*) *Description:* — A slightly opalescent liquid containing dead bacteria in suspension.

(*b*) *Identification:* — It gives specific agglutination when mixed with the serum of the animals infected with *S. abortus equi* organisms.

(c) *Sterility Test:* — Should comply with the test for sterility general monograph on «Diagnostic Antigens».

5. **Labelling and Storage:** — Should comply with the requirements of «Labelling» and «Storage» as laid down in the general monograph on «Diagnostic Antigens».

6. **Expiry Date:** — The date of expiry of potency shall be not more than nine months from the date of manufacture when stored at 20 C to 40 C.

SALMONELLA PULLORUM COLOURED ANTIGEN

1. **Synonym:** — Bacillary Dierrhoes (B.W.D.) Antigen.

2. **Definition:** — The antigen is a suspension in a solution containing 1 per cent Formaline, 1 per cent KH2 P04 and 0.85 per cent Sodium Chloride of pure smooth culture of a standard strain of *Salmonella Pullorum.*

3. **Preparation:** — Standard strain of **S. Pullorum** is grown on sulphur agar medium in Roux flasks for five days at 370° The pure growth is washed with 1.0 per cent Formol Saline.

STANDARDISATION

The antigenic cell suspension is then centrifuged (preferably in cold centrifuge) for half an hour at 4000 rotations per minute and the packed cell volume determined. The packed cell is then re-suspended in a solution containing 1% formalin, 1% KH2 P04 and 0.85% sodium chloride. 1 ml. of packed cell is suspended in 10 ml. of the resuspendiary solution, mixed throughly and is passed through a cotton wool pad. The turbidity of the antigenic suspension is usually between 100 to 125 times Mac Farland scale standard and optimum 3 c.c. of a 1 per cent aqueous solution of crystal violet are added to 100 ml. of the antigenic suspension. After making the dye the antigen is allowed to stand forty-eight hours before use. The average yield per Roux-flask of culture medium is about 50 ml. The antigen should be bottled in 10 ml. or 20 ml. quantity in amber-coloured bottles and corked with rugger caps and paraffin sealed and preserved until required for used within the expiry period. This antigen reacts instatly with the blood of all carrier birds and remains permanently negative with that of non-infected birds.

This antigens gives good reactions with positive sera whose litre is even as low as 1:20.

4. **Standard:**

(a) *Description:* — Violet coloured liquid containing dead bacteria in suspension.

(b) *Identification:* — It gives specific agglutination when mixed with the serum of birds infected with S. Pullorum infection. It is used for carrying out plate agglutination test for serological diagnosis for *S. Pullorum* infection in birds.

(c) *Sterility Test:* — Should comply with the test for sterility described in the general monograph on «Diagnostic Antigen». The tests shall be done before addition of «Crystal Violet».

5. **Labelling and Storage:** — Should comply with the requirements of «Labelling» and «Storage» as laid down in the general monograph on «Diagnostic Antigens».

6. **Expiry Date:** — A six month expiration date for this antigen is recommended. However, it is advisable to use fresh ones as far as possible. This antigen should be preserved at 4° to 6° in a dark place in the refrigerator and should not be exposed to hot weather condition for longer than necessary before use in the field.

SALMONELLA PULLORUM PLAIN ANTIGEN

1. **Synonym:** — Bacilary White Diarrnoes (B.W.D.) Plain Antigen.

2. **Definition:** — The antigen is a suspension of puro smooth culture of Salmonella Pullorum in phenol saline.

3. **Preparation:** — Forty-eight hours old pure culture of smooth strain of S. pullorum is washed with 0.5 per cent phenol saline and the pooled suspension is adjusted to contain approximately 800 million organisms per pl. by the addition of more carbol saline. The suspension is kept at room temperature for twenty-four hours, and dispensed in suitable containers.

4. **Standard:** —

(a) *Description:* — An opalescent liquid containing deed bacteria in suspension.

(b) *Identification:* — It gives specific agglutination when mixed with the serum of birds infected with *S. Pullorum.*

(c) *Sterility Test:* — Should comply with the tests for sterility described in the general monograph on «Diagnostic Antigens».

5. **Labelling and Storage:** — Should comply with the requirements of «Labelling» and Storage» as laid down in the general monograph on «Diagnostic Antigens».

6. **Expiry date:** — The date of expiry of potency shall be not more than nine months from the date of manufacture when stored at 2°C to 4°C.

TUBERCULIN

1. **Definition:** — (i) Tuberculines are preparations of fluid media on which Mycobacterium tuberculosis has been grown in artificial culture and which has been freed by filtration from the bacilli.

(ii) For the purposes of the Schedule tuberculinas are classified in (a) Tuberculine-subcutaneous (b) Heat concentrated synthetic Medium (H.C.S.M.) Tuberculine (c) Avian tuberculine.

2. **Preparation:** —

(a) *Tuberculine subcutaneous:* Flasks containing Henley and Dorset synthetic medium are inoculated with standards human strains of Myco. Tuberculosis previously grown on glycerol-beef broth medium for ten day. After ten to twelve weeks of incubation at 37°C flasks containing pure growth are steamed for three hours. The contents are filtered through fine meshed sieve and the volume is made up to its original with phenolised distilled water such that the final concentration of phenol is 0.5 per cent. It is then filtered through Seitz filter.

(b) *Heat Concentrated Synthetic Medium (H. C. S. M.) Tuberculine:* — To the strained liquid obtained after sieving as in the method of preparation of Tuberculine subcutaneous, glycerol is added in the proportion of 122 ml. per litre of the original volume of medium used. The mixture is evaporated to one-fifth of the original volume on a steam bath. An equal volume of 1 per cent phonol in distilled water is added after the mixture is cooled. The product is stored at 47°C for fourteen days before it is filtered through Seitz filter. It is then dispensed in ampoules.

(c) *Avian Tuberculine Concentrated:* — The procedure is the same as for Tuberculine Concentrated (H. C. S. M.). Except that standard strain of Myco-tuberculosis (Avium) is used in its preparation.

3. **Standard:** —

(a) *Description:* — A yellowish brown viscous liquid.

(b) *Identification:* — When injected intradermally into the animal infected with tuberculosis diffused swelling occurs depending upon the allergic status of the animal the magnitude of dose and specificity of the product. In non-infected animals this reaction is not observed.

(c) *Sterility Test:* — Should comply with the test for sterility described in the general monograph on 'Diagnostic Antigens'.

(d) *Potency Test:* — (1) *Tuberculine subscutaneous* Six large white guinea-pigs each weighing not less than 300-450 g. are individually inoculated intra-muscularly with 0.5 mg. (moist growth from solid flants) of *Mycobaeterium tuberculosis* three weeks prior to test of each batch of tuberculine. The following dilutions of (a) test tuberculine and (b) standard tuberculine are used: —

1 in 200, 1 in 400, 1 in 800, 1 in 1600.

The six sensitized guinea-pigs are depilated on one flank and after about twenty four hours each animal inoculated intradermally with 0.2 ml. of each dilution of the two tuberculines in two rows. The reactions are read after twenty-four and forty-eight hours. When the local reactions produced by the graded inter-dermal injections of the two preparations are comparable the brew is passed for issue.

(ii) *Heat Condentreated Synthetic Medium (H. C. S. M.) Tuberculiee:* Six adult white guinea-pigs each weighing not less than 300-450 g. and sensitized three weeks priviously with 0.5 mg. (moist growth from solid slants) of *Myco-Tuberculosis,* bovine type, injected intramuscularly are used for test of each batch. The following dilutions of (a) test tuberculine and (b) standard tuberculine are used: —

1 in 500, 1 in 1000, 1 in 2000 and 1 in 4000.

The six sensitized guinea-pigs are depilated in one flank and after twenty-four hours each animal is inoculated intradermally with 0.2 ml. of each dilution of the two tuberculines in two rows. The reactions are read after twenty-four and forty-eight hours. When the local reaction produced by the graded intradermic injections of the two preparations are comparable, the test tuberculine is passed for issue. The tuberculine is dispensed in ampoules.

(iii) *Avian Tuberculine:* — Six adult fowls, with well developed wattles, sensitized at least three weeks previously by intramuscular injections with 10 mg. moist weight (from solid slants) of twenty-one days old culture of Mycobacterium Tuberculosis (Avian Type) are used for potency test of each batch. In each fowl, one wattle is inoculated with 0.2 ml. of undiluted test tuberculine and the other wattle with similar quantity in each fowl are read after twenty-four hours and forty-eight hours and if comparable the product is passed for issue.

4. **Labelling and Storage:** — Should comply with the requirements of «Labelling» and «Storage» as laid down in the general monograph on «Diagnostic Antigens».

5. **Expiry date:** — The date of expiry of potency shall be not more than two years from the date of manufacture when stored at 2°C to 4°C.

PART IV — GENERAL

1. For the purposes of this Schedule any test or method of testing described in the British Veterinary Codex shall be deemed to be a method approved by the Licensing Authority.

2. The Licensing Authority shall publish in the Official Gazette from time to time particulars of any test or method of testing approved by him».

19. In the Schedule K of the said Rules, items 3 and 4 and the entries relating thereto shall be omitted.

Sd/-

S. P. JINDAL
Under Secretary

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